Penicillin Transformations

II.* Properties and Structural Determinations of 2,2-Dimethyl-3(S)-methoxycarbonyl-6-acylamino-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepines

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In the epimerisation reaction of methyl 6β-phthalimidopenicil-lanate (I) to the 6α-isomer (II) in methylene chloride in the presence of triethylamine, an additional compound, the 2,2-dimethyl-3(S)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiaze-pine (III) was isolated. After transformation into its phenylacetyl derivative (VI) the non-identity of VI with the known 5-oxo-1,4-thiazepine derivative XI was shown in a parallel experiment. The structure of III was established by spectral data and degradation into the authentic 2,4-dinitrophenylhydrazones of methyl 2-oxoisovalerate (XXIII) and O-methyl (XVI) and S-benzyl 2-phthalimido-2-formyl-acetate (XXIVa), respectively.

III was also predominantly produced together with a small amount of the 6α-epimer (II) from I by the action of POCl₃ in benzene under conditions which were used in an early penicillin synthesis. It was possible to convert the 6α-epimer (II) into the 7-oxo-1,4-thiazepine derivative (III) under similar conditions as used with I. The inter-pretation of the formation of the 7-oxo-1,4-thiazepine derivative (III) and of the general mechanism of the epimerisation at C(6) of the penicillin structure is discussed.

T he penicillin molecule is known to undergo a great number of degradation and rearrangement reactions. On the whole, the reactions appear to be initiated by an attack on the amide bond of the strained β-lactam ring which generally is considered to be the most reactive part of the molecule. Recently, however, reactions have been found which occur as a result of the special reactivity imparted to the molecule by the sulphur of the thiazolidine ring. In the penicillin structure several hydrogen atoms, at C(3), C(6) and in the dimethyl group, are situated in β-position with regard to the sulphur and may participate in β-elimination reactions leading to formation of an intermediate thiol structure. Such reactions generally require rather vigorous conditions,[1,2] but may proceed under relatively mild ones in the case of the penicillin mole-

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* Part I, *Tetrahedron Letters* **1969** 1863.

cule owing to additional activation of the hydrogen atoms by the side chain, the carboxyl group or by the oxidised sulphur. Examples of such reactions are anhydropenicillin formation,[3] the rearrangement of penicillin sulphoxides into Δ^3-cephems [4] and Raney-nickel desulphurization.[5]

Some penicillin structures have been found to undergo facile C(6) epimerisation under basic conditions, and Wolfe et al.[6,7] have proposed a β-elimination mechanism for the epimerisation of methyl 6β-phthalimidopenicillanate involving the hydrogen at C(6). Recently we have found [8] that an additional compound is obtained in this reaction. The formation of this compound requires the occurrence of such a β-elimination. The purpose of this paper is to describe the structure and properties of this compound and to discuss its possible role in the epimerisation reaction.*

On preparing methyl 6α-phthalimidopenicillanate (II) according to Wolfe and Lee [6] by treatment of I with triethylamine in methylene chloride at room temperature, TLC showed the formation of an additional product (III), which could be isolated in a 25 % yield.[8] The same substance was also obtained in a lower yield (12 %) from the 6α-isomer (II) on prolonged treatment (10 days) under similar conditions.[8] Similarly III was produced in good yield (57 %) if the 6β-isomer (I) was treated with phosphorus oxychloride [9] under the conditions used by Sheehan and Cruickshank [10] in the total synthesis of the penicillin ring system from the racemic α-isomer of 4-methoxycarbonyl-5,5-dimethyl-α-phthalimidothiazolidine-2-acetic acid (XXVIIIb). From this reaction we have also isolated the 6α-isomer (yield 4.1 %), but no substance identical with the starting material (I) could be detected (over 1 %).**
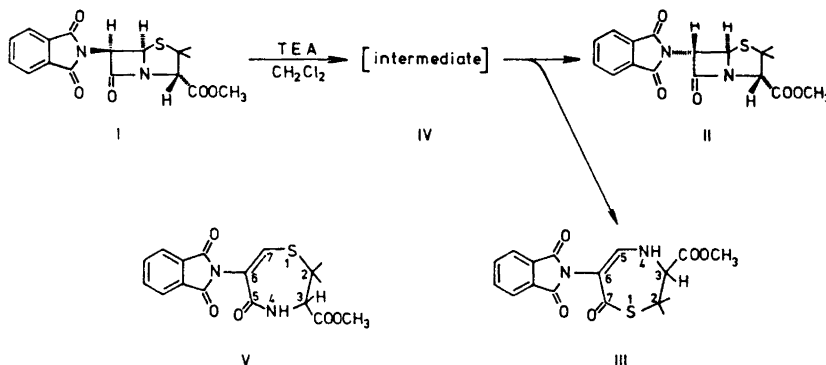
Scheme 1.

Elemental analysis and molecular weight determination of III showed it to be isomeric with I and II. Its UV and IR spectra were found to be strikingly similar to those reported by Sheehan and Cruickshank [10] for a compound (see

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* Main results and conclusions were briefly reported at the 10th European Peptide Symposium.[9]

** The observation of Clayton et al.[11] that I is recovered unchanged after the treatment with phosphorus oxychloride has not been confirmed.[12]

Table 1. Physical and chemical properties of 7-oxo-1,4-thiazepine (III) and 5-oxo-1,4-thiazepine (V)[10] derivatives.

	III	V[10]
Formula	$C_{17}H_{16}N_2O_5S$	$C_{17}H_{16}N_2O_5S$
M.p.	$217-218°C$	$237-237.5°C$
$[\alpha]_D^{20}$	$-215°$ (c 0.5; CH_3OH)	—
UV$_{CH_3OH}$ λ_{max}	304 mμ, $\varepsilon=11\,800$	304 mμ, $\varepsilon=12\,000$
	256 mμ, $\varepsilon=8\,350$	250 mμ, $\varepsilon=7\,700$
	238 mμ, $\varepsilon=13\,000$	238 mμ, $\varepsilon=13\,000$
	217 mμ, $\varepsilon=42\,900$	218 mμ, $\varepsilon=46\,000$
After desulphurization and acid hydrolysis	No alanine	—

Table 1) isolated as a by-product in a total synthesis of the penicillin ring system and assigned the structure of a 2,2-dimethyl-3-methoxycarbonyl-6-phthalimido-5-oxo-2,3,4,5-tetrahydro-1,4-thiazepine (V). In the IR spectrum of III the characteristic β-lactam absorption of the penicillins around 1770 cm^{-1} was missing but a broad absorption band with peaks at 1772, 1740, and 1710 cm^{-1} showed the presence of the phthaloyl and methyl ester group. It further contained a sharp band at 3440 cm^{-1}, indicating an NH-group and an absorption at 1652 cm^{-1} and a further one with double peaks at 1752 and 1550 cm^{-1} which Sheehan and Cruickshank had attributed to the vinyl sulphide and the amide bonds, respectively, in their 5-oxo-1,4-thiazepine structure. The NMR spectrum of III showed peaks corresponding to the phthaloyl, methyl ester, and the dimethyl groups in the expected position and contained in addition three multiplets coupling with each other. The appearance of the multiplets was, however, found to be solvent dependent. In deuterated dimethyl sulphoxide they consisted of two triplets each with an integral corresponding to one proton ($\tau=5.44$ and 2.70 ppm) and a quartet ($\tau=1.28$ ppm) with an integral corresponding to about one half of a proton. On addition of a drop of water to the test solution the triplets were converted into doublets ($\tau=5.44$ ppm, $J=6$ cps, $\tau=2.70$ ppm, $J=9$ cps) and the quartet, now with an integral corresponding to a full proton, could be recognised to consist of two doublets with $J=6$ and 9 cps, respectively.* By spin-decoupling it was found that the two doublets coupled with the quartet, but not with each other. That the three protons were thus forming an AMX-system was further confirmed by taking a spectrum in deuterated dimethyl sulphoxide after addition of deuterium oxide, whereby the triplets collapsed into singlets corresponding to the central peaks and the quartet disappeared. In dimethylacetamide the pure AMX-system was obtained whereas triplets were found when spectrum was run in deuterated pyridine or acetone.

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* In the spectrum originally published [8] the central peaks of the triplets were barely recognisable and were overlooked. The chemical shifts recorded were also slightly different from the ones now reported.

The coupling pattern found can be accommodated to the structure V only by assuming a long range coupling between the protons at N(4) and C(7). The NMR-spectrum of V has not been described, but in the spectrum of the corresponding phenylacetamido compound no such coupling is observed, the proton at C(7) appearing as a sharp singlet at $\tau = 2.34$ ppm [14] (Table 2). The spectrum instead suggested that our compound should have the 2,2-dimethyl-3-methoxycarbonyl-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine structure (III), the AMX-system being formed by the protons at C(3), C(5), and N(4), respectively. The observed solvent dependence of the spectrum could be explained by a partial dissociation of the proton at N(4). In agreement with this the compound III was found to be about as acidic as m-nitrophenol.

The other spectral data observed for the compound would appear to be in accordance with structure III. In IR the thiolester absorption, expected at $1660-1700$ cm^{-1}, could be represented by a shoulder at 1680 cm^{-1} of the broad absorption band corresponding to the phthaloyl and the methyl ester; absorptions at 1630, 1570, and 1550 could be attributed to the double bond and the NH-group. The UV-spectrum would appear consistent with the chromophoric system of III as well as with that of V. Further evidence for the thiol ester group in III was obtained from the mass spectrum which contained two peaks at $m/e = 300$ (relative intensity 10 %) and 60 (relative intensity 35 %) indicating the loss of COS from the molecular ion. The mass of the first one could be estimated by high resolution mass spectrometry and was found to correspond well to that of the expected ion (found $m/e = 300.1121$; calc. for $C_{16}H_{16}N_2O_4^+$, $m/e = 300.1110$). Both ions were absent or of very low intensity in the spectra of I and II.

The spectral data obtained thus indicated that the structure of III was different from that of V. On the other hand III was found to react with the same readiness with sodium methoxide as has been reported for 2,2-dimethyl-3-methoxycarbonyl-6-phenylacetamido-5-oxo-2,3,4,5-tetrahydro-1,4-thiazepine (XI),[13] which contains the same ring structure as V. In both cases the optical activity of the compound is rapidly lost and the reaction product reacts with benzyl bromide to give a benzyl derivative, which in the case of XI was identified as the dehydrovalinate structure XIII. In order to verify that III contained a ring structure different from that of V and XI it was found necessary to prepare the phenylacetyl analogue (VI) of our compound in order to directly compare its properties with those reported for XI. Treatment of III with hydrazine hydrate in aqueous dioxane for three days at $+4°C$ removed the phthaloyl group completely to give the corresponding derivative with a free amino group, which was not isolated but directly converted into the phenylacetyl derivative VI by treatment of the reaction mixture with phenylacetyl chloride. VI was found to have physical properties clearly differing from those reported for XI [13,14] proving that the two compounds were not identical (Table 2). In the NMR-spectrum of VI an AMX-coupling system was found corresponding to the one found for III indicating that the ring system of III had been preserved during the reactions. The compound (VI) was found to be hydrolyzed with sodium hydroxide in aqueous ethanol at about the same rate as XI (Fig. 1), and on treatment with methanolic sodium methoxide it lost its optical activity rapidly in the same manner as XI. Addition of

Table 2. Physical and chemical properties of the two 6-phenylacetamido thiazepine compounds (VI and XI [14]), the benzylthiocarbonyl derivative of VI (VIII), and the benzylthiovinyl derivative of XI (XIII). [13]

|	7-Oxo VI	5-Oxo XI [14]	Benzylthiocarbonyl VIII	Benzylthiovinyl XIII [13]
Formula	$C_{17}H_{20}N_2O_4S$	$C_{17}H_{20}N_2O_4S$	$C_{24}H_{28}O_4N_2S$	$C_{24}H_{28}O_4N_2S$
M.p.	150—151°	142.8—143°	110—112°	154—155°
$[\alpha]_D$	−195° (20°, c 1, chloroform)	−135° (25°, c 3.5, chloroform)	—	—
UV λ_{max}	204 mμ, ε=14 500; 258 mμ, ε= 6 400; 315 mμ, ε= 9 600 (in MeOH)	235 mμ, ε= 9 650; 305 mμ, ε= 5 250 (in EtOH)	—	299 mμ, ε= 15 700 (in EtOH)
IR(KBr) ν_{max}	1710 1620	1745 1660	1670, 1655 1590, 1495	1662, 1635 1512, 1325
NMR τ	2.06d (J=8.5), 2.33—−2.78, 5.40d (J=5.5), 6.14, 6.33, 8.37, 8.40 (in C_5D_5N)	2.34, 2.72, 2.86d (J=7), 5.82d (J=7), 6.21, 6.37, 8.48, 8.72 (in $CDCl_3$)	2.10, 2.66, 2.74, 2.80, 5.87, 6.23, 6.32, 7.88, 8.10 (in $CDCl_3$)	1.85, 2.03, 2.73, 6.02, 6.34, 6.41, 7.89, 8.25 (in $CDCl_3$)
After desulphurization and acid hydrolysis	No alanine	Alanine	—	—
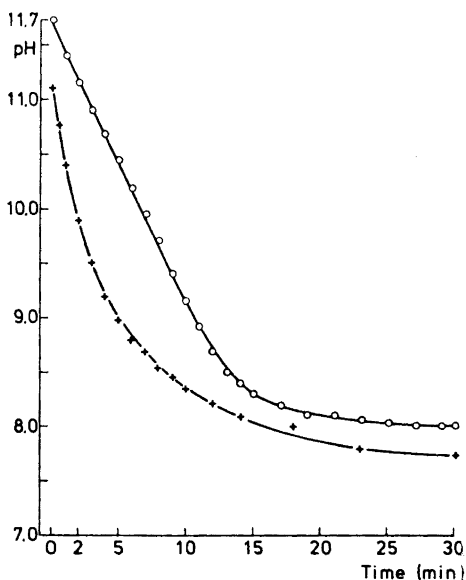
Fig. 1. Change of pH of reaction solution after treatment of 2,2-dimethyl-3(S)-methoxycarbonyl-6-phenylacetamido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (VI; ○) with half-equivalent amount of NaOH. The values for 2,2-dimethyl-3(S)methoxycarbonyl-6-phenylacetamido-5-oxo-2,3,4,5,-tetrahydro-1,4-thiazepine (XI; +) were taken from Leonard and Ning.[13]

benzyl bromide to the reaction solution containing VII readily gave a benzyl derivative (VIII) with properties clearly differing from those reported for XIII [13] (Table 2). In the case of XI, the reaction with sodium methoxide has been found to be a β-elimination over the C(2) − C(3) bond, starting with removal of the proton at C(3) and leading to the formation of an enethiolate-dehydrovalinate structure XII, which has been isolated as its sodium salt. The facility of the elimination has been explained by the stability of the enethiolate moiety in XII.[13] In the structure VI the corresponding reaction would lead to a carbothiolate-dehydrovalinate structure VII in which the carbothiolate group would appear to be at least as stabilized as the enethiolate in XII allowing the elimination reaction to proceed as readily as in the case of XI. The reaction of the carbothiolate (VII) with benzyl bromide would then lead to a thiobenzyl ester (VIII). All spectral and other properties of the reaction product were also found to be in accordance with such a structure. Whereas the elimination reaction proceeds with the same readiness in the two ring systems the consecutive reactions of the carbothiolate and the enethiolate with alkylating agents would proceed with different rates depending on differences in nucleophilicity of the two anions. With benzyl bromide no difference was noted, but whereas XII has been found to react very rapidly with 2,4-dinitrochlorobenzene in ethanolic solution at room temperature, VII was found to be virtually unreactive under these conditions.

The 5-oxo-2,3,4,5-tetrahydro-1,4-thiazepine structure in XI is readily broken down into two amino acids, alanine and valine, by Raney-nickel desulphurisation followed by acidic hydrolysis.[14] However, under the same conditions III and VI do not give any alanine at all and only trace amounts of
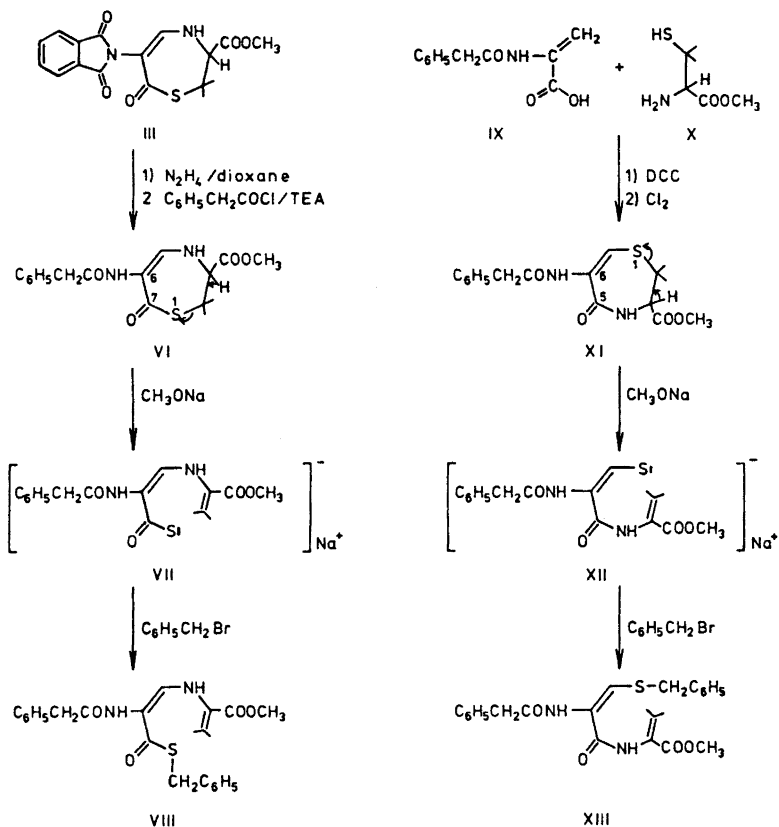
Scheme 2.

valine according to TLC in five different systems (Table 3). This is in good agreement with the 7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine structure adopted for III and VI where the reductive desulphurisation step would be expected to remove the thiolester group and give an N-alkylated valine structure which on hydrolysis cannot form any alanine, and valine only with difficulty. In the IR spectrum of the product of the desulphurisation step, the absorption bands corresponding to the double bond (1630 cm^{-1}) and the thiolester group of III (shoulder at 1680 cm^{-1}) were absent.

Whereas the previous described reactions only give evidence that III cannot have the ring structure adopted by Sheehan and Cruickshank [10] for their compound, direct proof for the 7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine ring was obtained in a study of the reactions and properties of the compounds originating from the methyl dehydrovalinate derivative formed in the reaction of III with sodium methoxide. The reactions and compounds are summarized in Scheme 3.

Table 3. TLC analysis of the degradation products of III and VI after desulphurization and hydrolysis.

| Mobile phases | hR_F Values | | | | |
| | On Silica gel G | | | On Cellulose F | |
	M	K	J	N	K
DL-Alanine	11	23	48	22	20
D-Valine	17	36	56	40	43
Main spot from III	15	18	14	43	28
Other spots from III	—	40*	55*	—	40*
Main spot from VI	14	18	13	42	28
Other spots from VI	61 34 19 16*	36* 27 8	65 55* 47 34	50 36*	42* 23

III was found to react in the same manner with sodium methoxide as the corresponding phenylacetamido derivative (VI). The elimination reaction proceeded very rapidly with complete loss of the optical activity of the reaction solution. The carbothiolate-dehydrovalinate compound (XIV) was not isolated but treated directly with two equiv. of hydrogen chloride in dry methanol at room tempreature for four days to give a mixture of the methyl ester XVII and the thiomethyl ester XVb. This is in accordance with the findings that carbothiolic acids on esterification with low molecular weight primary alcohols give mixtures of O- and S-alkyl esters, the former predominating. [15] Hydrolysis of the reaction product in 97.5 % methanol containing hydrogen chloride and 2,4-dinitrophenylhydrazine gave a mixture of three 2,4-dinitrophenylhydrazones, two of which, XVI and XXIII, could be isolated in pure form by chromatography on a silica gel column, the third one probably being the thiomethyl compound XXIVb. By comparison with authentic samples prepared according to the literature, XVI and XXIII were identified as the 2,4-dinitrophenylhydrazones of methyl 2-phthalimido-2-formylacetate [16] and methyl 2-oxoisovalerate, respectively. [17] This showed that the bis-enamine structure in XVII could be hydrolysed with release of the two oxo derivatives forming it.

The ambiguity of the esterification reaction could be avoided by going *via* the thiobenzyl ester (XVa) prepared by reaction of III with sodium methoxide in dry methanol followed by benzyl bromide. The compound was found to exist in at least two isomeric forms (see below), but after recrystallization from 75 % dioxane containing hydrogen chloride it was obtained as a homogeneous compound with analytical and spectral data in accordance with the adopted structure (XVa). The presence of an NH group was shown by a medium absorption at 3320 cm^{-1} in the IR spectrum and in the NMR spectrum (in deuteriochloroform) by a doublet at $\tau = -0.12$ ppm coupling with the adjacent vinylic proton ($\tau = 3.24$ ppm). The isopropylidene group was indicated by two singlets at $\tau = 7.98$ and 7.87 ppm. Hydrolysis of XVa in 97.5 % methanol containing hydrogen chloride and 2,4-dinitrophenylhydrazine for two days at room temperature gave a high yield of two 2,4-dinitrophenylhydrazones, which

a :   R = C₆H₅CH₂−
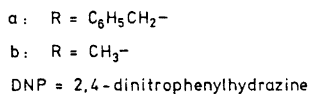
Scheme 3.

were separated and isolated by chromatography on a silica gel column. One was identical to **XXIII** whereas analytical and spectral data of the other one suggested that it was the 2,4-dinitrophenylhydrazone of *S*-benzyl-2-phthalimido-2-formylthioacetate (**XXIVa**). This was verified by preparing the compound in an independent way (Scheme 4) starting from *O*-benzyl 2-phthalimido-2-formylacetate (**XVIII**).[18] The latter was converted into its diethyl acetal

(XXV) and hydrogenated to give the free acid XXVI, which *via* the acid chloride or the mixed anhydride gave the thiobenzyl ester which then on hydrolysis in 97.5 % methanol containing hydrogen ˙chloride and 2,4-dinitrophenylhydrazine gave a 2,4-dinitrophenylhydrazone in all respects identical to the compound originating from the thiazepine (III).

The methylthiocarbonyl derivative (XVb) was obtained by treatment of XIV with methyl iodide under conditions analogous to those used for the preparation of the benzyl thiocarbonyl derivative (XVa). The NMR spectrum showed that it was a mixture of two forms (see below) but on hydrolysis in the presence of 2,4-dinitrophenylhydrazine it was converted in good yield into the two 2,4-dinitrophenylhydrazones XXIII and XXIVb. If the hydrolysis of XVa was carried out in the absence of 2,4-dinitrophenylhydrazine the *S*-benzyl 2-phthalimido-2-formylthioacetate (XXa) was formed. The latter could not be isolated in crystalline form but was directly converted by treatment with 2,4-dinitrophenylhydrazine into XXIVa identical to the compound previously obtained, and into its diethyl acetal (XXVII), which also was obtained from XVIIIa [18] (Scheme 4).
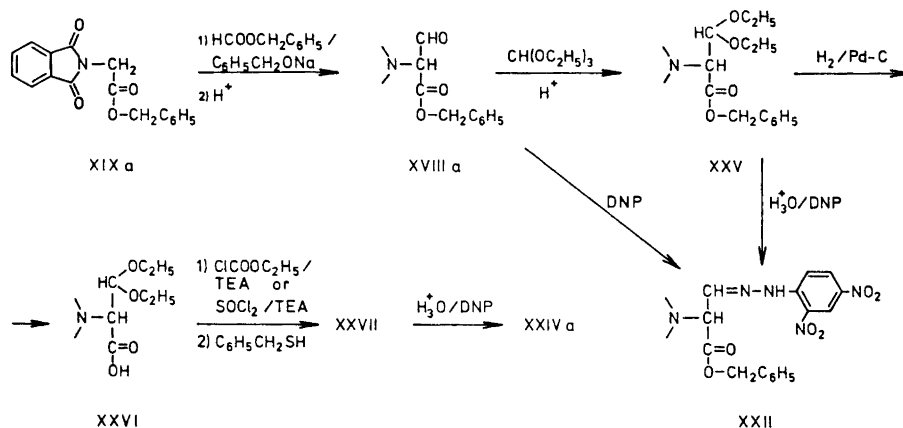
Scheme 4.

Esters of thiolcarboxylic acids are readily desulphurised by treatment with mercury salts. [19] When XVa was treated at room temperature with an equivalent amount of mercuric acetate in dry methanol it was converted in good yield into methyl *N*-(2′-phthalimido-2′-methoxycarbonyl)vinyl-2,3-dehydrovalinate (XVII) under concomitant formation of benzylthiomercuric acetate. XVII showed spectral properties similar to those of XVa with, *e.g.*, the same AX-system in the NMR spectrum, and chromatographic properties identical to those previously found for the product obtained by esterification of the carbothiolic acid XXI, but not isolated. Upon acid hydrolysis of compound XVII, methyl 2-phthalimido-2-formyl acetate (XVIIIb) was formed, identical in every respect to an authentic sample prepared from methyl

phthalimidoacetate (XIXb).[18] XVIIIb gave the 2,4-dinitrophenylhydrazone XVI identical to the product previously isolated in the preliminary hydrolysis experiment done with XVII in the presence of 2,4-dinitrophenylhydrazine.

The reactions of Scheme 3 thus show that the carbon atoms in III are arranged in one methyl isovalerate and one α-phthalimidoacrylate structure, which are linked to each other both through a thiolester bond and through an amino group in an enamine structure as required by the structure adopted for III. That III also can be obtained by treatment of I with phosphorus oxychloride, the cyclizing reagent used by Sheehan and Cruikcshank [10] in their penicillin synthesis, suggests that the by-product they regarded as a 5-oxo-1,4-thiazepine (V) rather should have the 7-oxo-1,4-thiazepine structure (III). That this really is so was recently verified by Clayton et al.[11] in the repetition of the original experiment. Furthermore, as the methyl 6α-phthalimido-penicillanate (II) is formed and the 6β-isomer (I) is completely consumed when the latter is treated with phosphorus oxychloride under the conditions used by Sheehan and Cruickshank, it is possible that the penicillin compound they isolated actually had the 6α-structure.[9]

Very recently further examples of the formation of 7-oxo-1,4-thiazepine derivatives have been published.[11,20-22]

The double bond system of the methyl N-[(2'-phthalimido-2'-carbonyl)-vinyl]-2,3-dehydrovalinate structures XV and XVII may exist in isomeric forms. Although the products obtained gave analytical figures in excellent agreement with the adopted structures, they gave two spots on TLC on inactive, buffered (pH 6.6) plates. By column chromatography or by recrystallization in the presence of hydrogen chloride it was possible in the case of XVa and XVb to isolate the main components which according to spectral data had a bis-enamine structure as shown in Scheme 5.* If the homogeneous com-

Scheme 5.

pounds were chromatographed on active, unbuffered silica gel plates they appeared, however, as the same mixtures of two well separated components (Table 4) previously obtained. The phenomenon was studied more closely in the case of the thiobenzyl compound (XVa). The reaction solution obtained after treatment of III with sodium methoxide followed by benzyl bromide was investigated by TLC on buffered (pH 6.6) plates and found to contain in addition to XVa a second compound, XVaⁱ, present in a larger amount than

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* In the formulae of XV and its analogues (VII, XIV, XVII, and XIII, resp.) only one of the possible geometrical isomers is given.
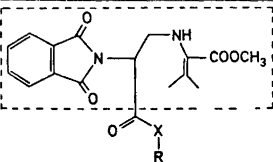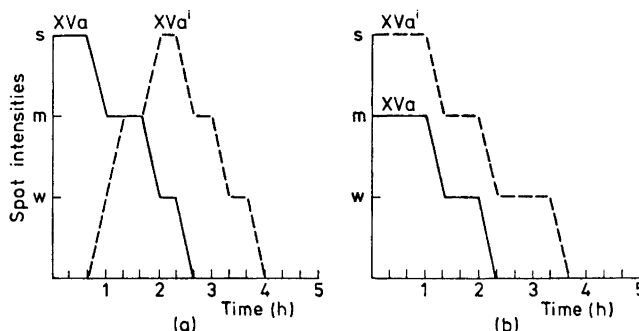
Fig. 2. Change of TLC* spot intensities on acidic hydrolysis of (a) pure XVa and (b) a mixture of XVa and XVaⁱ, resp.

the former. However, evaporation of the solution caused a change in the relative amounts of the two compounds so that XVa was now the main product in the residue. If, however, the reaction solution was *not* evaporated but treated with methylene chloride, washed with water and dried, the ratio of the amounts of the two compounds was preserved and they could be separated by chromatography on a silica gel column. XVaⁱ was found to be stable in solution at +5°C for several days, but all operations at +20°C led to partial conversion into XVa and to formation of decomposition products. That the two compounds were interconvertible and thus were isomers could be demonstrated by thin layer chromatography in several experiments and the transformation was found to be catalyzed by the active silica gel surface of the chromatographic plates and by sodium methoxide. Some information about the structure of XVa could be gained from the IR and NMR spectra taken on solutions of the compound in chloroform and deuteriochloroform, respectively, prepared from the chromatographic eluates by exchange of solvents at low temperature. It appeared from the NMR spectrum that the isopropylidene group of XVa ($\tau = 7.98$ and 7.89 ppm) was present in XVaⁱ too ($t = 8.02$ and 7.88 ppm) and that instead the acrylate part of the molecule had been changed, as the doublets ($J = 13$ cps) corresponding to the vinylic proton ($t = 3.24$ ppm) and the amino proton ($\tau = 0.12$ ppm) of XVa were missing. In the IR spectrum of XVa the band at 1650 cm^{-1}, probably corresponding to the unsaturated benzyl carbothiolate moiety, had disappeared. Acidic hydrolysis of XVa appears to proceed through XVaⁱ as shown by a comparison of the relative intensities of the TLC spots given by XVa and XVaⁱ during the hydrolysis (Fig. 2). We tentatively suggest XVaⁱ to have the methyl *N*-(2′-phthalimido-2′-benzylthiocarbonyl)ethylidene-2,3-dehydrovalinate structure.

Methyl 6α-phthalimidopenicillanate (II), on treatment with triethylamine under conditions identical to those employed with I, was also found to give III, although at a considerably slower rate and to a lesser extent. In this reaction compound I could not be detected. Treatment of III in a similar way gave neither I nor II. This suggests, however, that the two reactions, the epimerisation and the thiazepine formation, should have a common inter-

Table 4. hR_F values of methyl N-[(2'-phthalimido-2'-carbonyl)vinyl]-2,3-dehydro-valinate derivatives on pre-coated unbuffered and inactive buffered plates.[a]

	= DV	Solvent systems	Silica gel plates	
			F_{254} pre-coated (Merck)	$HF_{254+266}$ (Merck) 0.5 mm, made with McIlvaine's pH 6.6 buffer solution
DV–C=O | S | CH₂ | C₆H₅	XVa	E	43 and 23	51
		N	37 and 11	30
DV–C=O | S | CH₃	XVb	E	40 and 21	44
		N	33 and 10	24
DV–C=O | O | CH₃	XVII	E	36 and 16	40
		N	30 and 6	15

[a] Plates were stored for 2 h after applying sample at room temperature and atmosphere, then developed.

mediate which is also supported by an analysis of the thermodynamics of the reaction.[7] The considerable difference in the specific rotation of I ($[\alpha]_D^{20}$ $+280°$) and its rearrangement product II ($[\alpha]_D^{20}$ $+207°$) and III ($[\alpha]_D^{20}$ $-129.5°$) provides a means of following the reaction in methylene chloride at 20° with varying ratios of I to triethylamine (Fig. 3). It can be seen that in all reactions, the rotations tend to reach the same end value suggesting that the product composition from the reaction is largely independent of the concentration of the base. On the other hand, it is evident that although tri-ethylamine only acts as a catalyst, the rate of the reaction increases with increasing base concentration (Fig. 3), indicating that the reaction involves the formation of a complex between the penicillin ester and the base, the formation or decomposition of which is rate-determining. Furthermore Wolfe and Lee [6] have shown that when the reaction is carried out in the presence of t-BuOD, no deuterium is incorporated into the epi-compound II. In our view the experimental facts are best accommodated in a reaction mechanism according to Scheme 6 involving the formation of an intimate ion-pair be-tween I and triethylamine (IV-A) which subsequently undergoes rearrange-ments leading to the epi-ester and the thiazepine.

The thiazepine (III) can arise only by internal acylation of the thiazolidine sulphur by the β-lactam carbonyl and this reaction can occur only after the C(5)–S bond has been broken. The formation of III is thus good evidence for
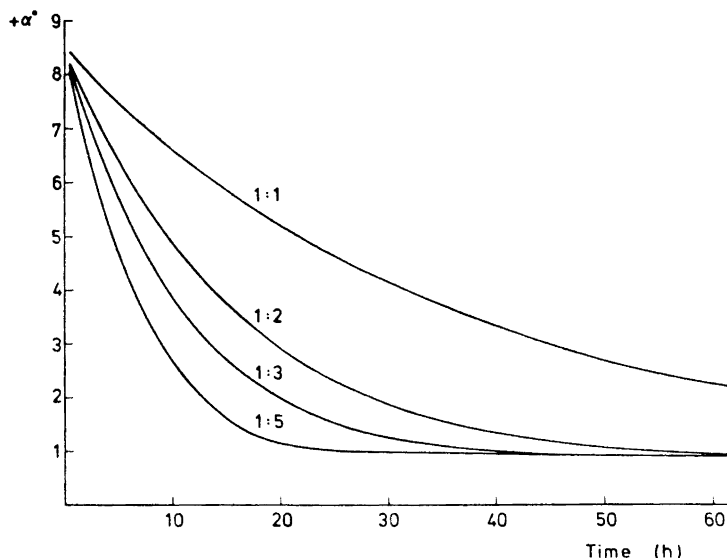
Fig. 3. Change of optical rotation (α) on treatment of methyl 6β-phthalimidopenicillanate (I) with different amounts of triethylamine in methylene chloride. (I: TEA ratios $= 1:1$, 1:2, 1:3 and 1:5.)

the existence of the ene-thiolate structure (IV-C) as a secondary intermediate, which would be obtained from IV-A in a β-elimination reaction, more particularly in accordance with an (E1cB)ip reaction.[23-26]

For the epimerisation several mechanistic pathways appear possible. One goes *via* the enolized structure (IV-D) which would be obtained from IV-A in a "conducted tour" mechanism where the negative and positive charges are not separated but displaced together. This type of mechanism, which also could apply to the ene-thiolate (IV-C) path, has been proposed for other isomerization reactions [27] and very recently Mayers and Kovacs suggested it for the racemization of protected cysteine esters in non-polar solvents.[28] A trialkylsilyl derivative of IV-D has been suggested as an intermediate in the epimerisation of esters of phenoxymethyl penicillin-(S)-sulphoxide by treatment with N,O-bis(trimethylsilyl)acetamide (BSA).[29] Very recently Vlietinck *et al.*[22] were further able to convert phenoxymethylpenicillin benzylester, which had been silylated with BSA into a 1:1 mixture of the 6α-isomer and the corresponding 7-oxo-1,4-thiazepine derivative under conditions similar to those used by Wolfe and Lee.[6] The mechanistic explanation of this experiment would be in good agreement with Scheme 6. In the epimerisation reaction IV-C and IV-D could be converted into the ion-pair IV-B where the carbanion has a C(6)-configuration opposite to that found in the penicillins and in IV-A. This configuration would be thermodynamically favoured depending on relief of the steric compression between the $6(\beta)$-side chain and the C(2) geminal dimethyl group in the penicillin structure.[30,31] This relief of

compression may, however, also be the driving force for the third possible pathway of the epimerisation, the direct transformation of IV-A into IV-B. The carbanions of the two ion-pairs probably having pyramidal configurations with sp^3-character should well be capable of undergoing inversion.[27] The rapid and quantitative transformation of methoxymethyl 6-p-nitrobenzylidenimino-penicillinate into the corresponding 7-oxo-1,4-thiazepine derivative without formation of the 6α-epimer [20] shows the important role of the 6β-substituent in the establishment of the equilibrium between the intermediates; i.e. the azomethine linkage promotes the ene-thiolate structure (IV-C) through conjugation.
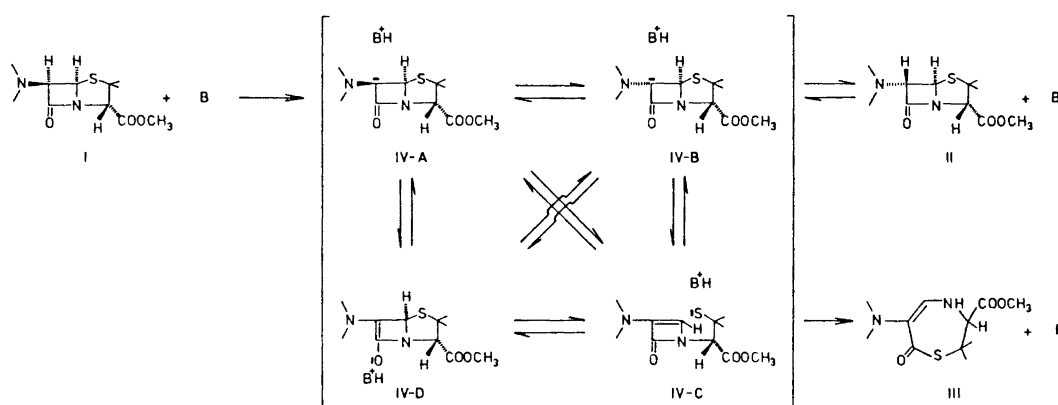
Scheme 6.

The experimental data so far available for the reaction do not allow any distinction between the various mechanistic possibilities.[32] From other closely related reactions some facts may, however, be gained which seem to disfavour the ene-thiolate as a dominating intermediate in the epimerisation. It has thus been found that an isolated β-lactam structure, cis-1,2-diphenyl-3-phthalimido-azetidinone, where a β-elimination reaction mechanism would appear to be excluded, is irreversibly converted into the trans-isomer when treated under conditions similar to those used in the present case.[33] Further, it has been shown by means of deuteration experiments that in the presence of aqueous base, simple de- and reprotonation of an intermediate carbanion explains the experimental results without any need for assuming a β-elimination step.[30,34] For this and other epimerisation reactions where bases stronger than triethyl-amine (OH⁻, NaH, NaNH₂, Bu^tO⁻) are used, an analogous mechanism may apply, involving not ion-pairs but solvated carbanions, which in principle could undergo the same rearrangements as the ion-pairs in Scheme 6. However, thiazepines (III) are not obtained in these reactions, indicating that structures corresponding to IV-C resulting from β-eliminations are not formed and thus that they are not necessarily intermediates in the epimerisation reaction under basic conditions.

More recently Ramsay and Stoodley have also proposed on very good experimental grounds the E1cB mechanism for the epimerisation reaction *via* an "anionic" intermediate.[21] It was postulated that the conversion of this intermediate into the ene-thiolate (IV-C) is the rate determining step of the 7-oxo-1,4-thiazepine formation, but that the ion-pairs (IV-A and IV-B, resp.) are more appropriate models for the epimerisation itself in the presence of weak bases. In apparent contrast to the results of Wolfe *et al.*[7] they found that the epimerisation of I with 1-methylpiperidine in CD_3SOCD_3 containing 5 % D_2O proceeded with deuterium exchange in the 6-position. This fact strongly supports the E1cB mechanism in that particular case, but in a solvent with less ionizing power like the dichloromethane used by Wolfe and Lee and by us the (E1cB)ip mechanism would still seem to be preferable.

A mechanism similar to that discussed above can account for the I→II + III transformation catalyzed by phosphorus oxychloride. In this case a bipolar complex ($POCl_2^+ \rightleftharpoons POCl_4^-$) originating from auto-ionization of the oxyhalide [35] may function as an acceptor of the proton at C(6) and at the same time make the sulphur involved in the β-elimination more electron attracting. This could explain the predominant formation of III (57.3 %) over II (4.1 %) in our experiment. The reactions catalyzed by antimony pentachloride can be interpreted in a similar way. However, other mechanisms are possible and for the latter reactions an attack of the inorganic halide on the amide bond of the β-lactam ring has been suggested [11] to form an acid chloride which could give III by acylation of the thiazolidine sulphur.

EXPERIMENTAL

All melting points were determined on a melting point apparatus according to Tottoli (W. Büchi Glasapparatefabrik, Flawil, Switzerland) and are uncorrected. All evaporations were carried out using a rotary evaporator at a pressure of 10 – 12 mm and a bath temperature of 24° except where stated otherwise. The symbol " $\rightarrow$ " represents a two-component solvent system, the composition of which changes in the direction of the arrow during the recrystallisation (continuous addition of the second solvent and slow simultaneous distillation in a rotary evaporator at 24° and 10 – 100 mm pressure). Whatman Phase Separating Paper No. 1 PS was used for predrying of organic extracts.

Mobile phases used in thin layer and column chromatography:

A = benzene-acetone (9:1)
B = chloroform-benzene (1:1)
C = chloroform-benzene (7:3)
D = chloroform containing 0.7 % ethanol
E = isopropyl ether-chloroform (7:3)
F = acetone-carbon tetrachloride-ethanol (5:4:1)
G = butanone-acetic acid-water (8.5:0.5:1)
H = chloroform-benzene-acetone (6:2:2)
I = carbon tetrachloride-isopropyl ether-acetone (5:4.5:0.5)
J = 96 % ethanol-water (7:3)
K = butanol-acetic acid-water (4:1:1)
M = chloroform-methanol-17 % ammonium hydroxide (2:1:1)
N = butanol-acetone-diethylamine-water (10:10:2:5)

Analytical thin layer chromatography was carried out on Silica Gel_{254} F precoated TLC plates (Merck) except where stated otherwise. The symbol "TLC*" represents analysis on self-prepared 0.5 mm Silica Gel $HF_{254+366}$ (Merck) plates on glass with

McIlvaine's pH 6.6 buffer solution. Detection was made under UV light except where stated otherwise. The intensity of the spots are noted as strong (s), medium (m), weak (w), or very weak (vw); this symbol supplemented with a formula-number, if the spot was identified by running standard compounds along with it. hR_F values [36] of non-identical standards are mentioned *after* the data for the investigated samples. For column chromatography Silica Gel $0.05 - 0.2$ mm (Merck) was used and packed by the suspension technique in dry benzene except where stated otherwise. "Two component" gradient elutions were achieved by the concave mixing of the first solvent (usually benzene) with the second solvent or with a solvent mixture. Subsequently there are also noted the total volume of the gradient period and the fraction volume. The fractions collected were checked by TLC.

The infrared spectra were recorded on a Unicam SP 200 or a Perkin-Elmer Model 21 recording spectrophotometer with potassium bromide discs or with solutions and the positions of the absorption maxima are expressed in wave numbers (cm^{-1}). The UV absorption spectra were recorded on a Unicam SP 800 recording and a Carl Zeiss PMQII spectrophotometer, respectively, and the positions of the absorption maxima or shoulder are expressed in nanometers (nm) followed by the corresponding extinction coefficient. The NMR spectra were recorded on a Varian A-60A spectrometer equipped with a V-6040 variable temperature controller and a spin decoupler Model V-6058A. The chemical shifts are given in τ-units using TMS or DDS as internal standards. Mass spectra recorded on an LKB 9000 instrument were obtained from the Mass Spectrometry Laboratory, Karolinska Institutet, Stockholm, Sweden. The most abundant ions are tabulated, followed by the relative abundance in per cent of the base peak. The specific rotations were measured on a Perkin-Elmer polarimeter 141 at 589 nm and the changes of the $[\alpha]_D$ values in the time were recorded with a coupled Servogor RE511 compensation recorder. The elemental analyses were performed by Alfred Bernhardt Microanalytical Laboratory, Elbach, Germany.

Methyl 6β-phthalimidopenicillanate (I). The compound was prepared according to Sheehan and Henery-Logan,[18] m.p. $174 - 175°$, $[\alpha]_D^{20} + 288.5°$ (*c* 2, chloroform), $+275.3°$ (*c* 2, acetone), $+279.7°$ (*c* 2, methylene chloride); (lit.[6] $[\alpha]_D + 279°$, chloroform; lit.[18] $[\alpha]_D^{28} + 288°$, butyl acetate).

IR (KBr): strong broad absorption band with peaks at 1784, 1764, 1736, 1718. NMR (CDCl$_3$): 8.49, 8.16 (2 s, 2×3H, C(CH$_3$)$_2$), 6.17 (s 3H, COOCH$_3$), 5.30 (s, 1H, CH), 4.38, 4.27 (2 d, $J = 4$, 2H, CH $-$ CH), 2.15 (m 4H, phthaloyl). MS: 187 (18), 174 (35), 132 (98), 114 (24), 104 (100), 99 (20), 76 (50), 59 (22). Molecular ion: 360.

Treatment of methyl 6β-phthalimidopenicillanate (I) with triethylamine in dichloromethane.[6] *Isolation of methyl 6α-phthalimidopenicillanate (II) and 2,2-dimethyl-3(S)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (III).* Methyl 6β-phthalimidopenicillanate (36.04 g, 0.1 mol) was treated in dry methylene chloride (720 ml) with dry triethylamine (30.3 g, 0.3 mol) for 12 h at 20°. The yellow solution obtained was evaporated *in vacuo* (20°) to a volume of about 200 ml, dry benzene (200 ml) was added and the mixture evaporated to dryness. Repeated evaporation with several portions of benzene afforded a yellow foamy residue which was dissolved in acetone (720 ml), treated with charcoal in the cold (1 h), filtered and concentrated *in vacuo* with continuous addition of methanol to give a crystalline precipitate which was filtered and washed with cold methanol. The white product (25.22 g, 71.1 %), m.p. $161 - 167°$, showed two spots on TLC (in system *H*): hR_F 52 (II) and 22 (III). Careful washing with cold methylene chloride (2×50 ml) gave a residue (10.1 g, 27.7 %; m.p. $217 - 218°$) which after recrystallization from acetone $\rightarrow$ methanol yielded the pure 2,2-dimethyl-3(*S*)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (III) (8.9 g, 24.7 %; m.p. $218 - 219°$). Analytical samples were obtained by recrystallization from methanol or from dry acetone-ether,[10] m.p. $220 - 221°$, $[\alpha]_D^{20} - 217.4°$ (*c* 0.5, methanol); $[\alpha]_D^{20} - 198.9°$ (*c* 2, acetone); $[\alpha]_D^{20} - 201.8°$ (*c* 2, pyridine); $[\alpha]_D^{20} - 129.5°$ (*c* 0.2, methylene chloride). The compound dissolved in pyridine was titrated potentiometrically with tetrabutylammonium hydroxide using a Radiometer R titrigraph SBR 2d connected through a TTT11 automatic titrator.

UV (methanol): 304(11 800), 256(8 350), 238(13 000), 218(42 900). IR (KBr): 3440m, 1780m, 1740s, 1710s, 1680sh, 1630s, 1570s, 1550s. *NMR (CD$_3$)$_2$SO*: 8.36, 3.82 (2 s, 2×3 H, C(CH$_3$)$_2$), 6.20 (s 3H, COOCH$_3$), 5.42 (d, $J = 6.14$, CH), 2.68 (d, $J = 9$, 1H, CH), 2.03 (s, 4H, phthaloyl), 1.32 (2 d, $J = 6$ and 9, 1H, NH). MS: 360 (20, molecular ion),

332 (17), 187 (37), 174 (100), 160 (17), 140 (40), 132 (20), 114 (22), 112 (22), 104 (41), 76 (28), 60 (32). (Found: C 56.83; H 4.47; N 7.92; O 22.42; S 8.81. Equiv. weight: 354. Calc. for $C_{17}H_{16}N_2O_5S$ (360.41): C 56.65; H 4.47; N 7.77; O 22.20; S 8.90.)

The filtrate, obtained on washing the mixture of the two isomers with methylene chloride, contained the 6α-ester (II) and a little of the thiazepine compound (III) according to TLC analysis (in H). After evaporation of the solvent (15.8 g, 43.8 %) and subsequent recrystallization of the residue from methylene chloride →— methanol, pure methyl 6α-phthalimidopenicillanate (II; 14.1 g, 39.2 %) was obtained, m.p. 182−183° (lit.[6] 183°), $[\alpha]_D^{20}$ +211.2° (c 2, chloroform), +207.6° (c 2, acetone), +207.4° (c 2, methylene chloride); (lit.[6] $[\alpha]_D^{20}$ +228°, chloroform).

IR (KBr): strong absorption band with peaks at 1760, 1735, 1720. NMR (CDCl$_3$): 8.50, 8.32 (2 s, 2 × 3H, C(CH$_3$)$_2$), 6.18 (s, 3H, COOCH$_3$), 5.33 (s, 1H, CH), 4.50, 4.39 (2 d, $J=2$, CH−CH), 2.14 (m, 4H, phthaloyl). MS: 246 (17), 187 (70), 174 (100), 172 (17), 161 (16), 160 (28), 132 (95), 114 (53), 104 (90), 99 (33), 87 (22), 76 (50), 75 (33), 53 (17), 50 (17), 46 (18), 41 (15), 28 (31). Molecular ion: 360.

In a repeated experiment with the same concentrations of I and triethylamine as above and carried out at $20 \pm 0.1°$ the isomerization was followed by semiquantitative TLC analysis. Aliquot samples (0.1 ml) were taken every 30 min, diluted with isopropyl ether (15 ml) and washed with 0.025 N hydrogen chloride (2 ml) and water (2 × 2 ml). The organic phase was filtered and evaporated to dryness. The residue was dissolved in methylene chloride (1 ml) and samples (5 μl ∼ 25 μg) were applied to 20 × 20 cm silica gel plates together with standards of II or III in increasing amounts. The standards were applied in between the test spots on a line 1 cm below (III) or above (II) the start line. For detection, the plates were sprayed with concentrated ammonia, stored over this reagent in a chamber for 2 h, dried, exposed to iodine vapor for 10 min and finally sprayed with aqueous starch-potassium iodide solution (1 %).[37] In mobile phase H (III as standard): hR_F 52 (I + II) and 21 (III), and in I (II as standard) after 4 consecutive developments: hR_F 52 (I), 47.0 (II) and 2 (III) were recorded.

The 6β-ester (I; sensitivity 2μg) could not be detected after 9 h, whereas the 6α-ester (II; sensitivity 2 μg) began to appear after 3.5 h and the thiazepine compound (III; sensitivity 0.6 μg) after 1 h. After 10 h further products could be detected. TLC analysis after 12 h: hR_F 38 (w) and 0 (w); after 36 h: hR_F 44 (w), 38 (w) and 0 (s); after 96 h: hR_F 44 (s), 38 (w) and 0 (s).

The isomerization of I was also followed polarimetrically at $20° \pm 0.1°$ varying the ratio I:triethylamine from 1:1 to 1:2, 1:3, and 1:5, respectively (Fig. 3).

Treatment of methyl 6α-phthalimidopenicillanate (II) with triethylamine in methylene chloride. Isolation of 2,2-dimethyl-3(S)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (III). Methyl 6α-phthalimidopenicillanate (3.6 g, 0.01 mol, recrystallized three times from methylene chloride →— methanol) was treated with dry triethylamine (3.03 g, 0.03 mol) in dry methylene chloride (72 ml) at 20° for 240 h. After 19 h the thiazepine compound (III) was detectable on TLC (in H): samples (1 μl ∼ 50μg) were applied directly from the reaction mixture: hR_F 53 (II) and 22 (III). Semiquantitative TLC analysis using a two-dimensional technique (first direction in H; second direction in I (twice); standard solutions were applied *after* development in H) showed the following conversions into III (%/h): 1.2/24; 2.5/48; 4/72; 7/120; 10/168; 14/240. Formation of the 6β-ester (I) could not be demonstrated by TLC. During the 10 days the $[\alpha]_D^{20}$ value of the reaction mixture changed from +212° to +131.6°.

Working up as described for the isomerization of I gave an acetone-insoluble white crystalline material (660 mg), m.p. 172−175°, after recrystallization from ethanol-ether, 184−186°. On the basis of m.p. and microanalytical data, the substance is most probably identical to chloromethyltriethylammonium chloride.[38] (Found: C 45.35; H 9.42; N 7.88; Cl 37.57. Calc. for $C_7H_{17}NCl_2$ (186.14): C 45.17; H 9.21; N 7.53; Cl 38.09.) IR (KBr): 3000m, 2640m, 2500w, 1630w, 1475 m.

The acetone solution was evaporated with continuous addition of methanol, finally reduced to a small volume, filtered and washed with cold methanol to give 2.25 g (68.3 %) of unchanged starting material (II), m.p. 182−183°, $[\alpha]_D^{20}$ +210.8° (c 2, chloroform), containing a small amount of III (< 0.5 % according to TLC in H). The mother liquor contained five substances readily detectable by TLC (in H): hR_F 63 (w), 54 (m, II), 48 (m), 24 (s, III) and 0 (m). The last compound was identical to the acetone insoluble substance above according to TLC (in G, hR_F 7). The mother liquor was evaporated,

dissolved in mobile phase H (5 ml) and chromatographed on a silica gel column (40 g; gradient elution: benzene → mobile phase H; 400/10 ml). Fractions 29 – 35 contained the thiazepine compound (III; 430 mg, 11.9 %) after recrystallization (acetone →–methanol, 190 mg), m.p. 218 – 219°, identical to authentic III by mixed m.p. and IR.

Attempted isomerisation of the thiazepine compound (III) with triethylamine in methylene chloride. 2,2-Dimethyl-3(S)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (III, 90 mg, 0.25 mmol) was stirred in dry methylene chloride (1.75 ml) containing dry triethylamine (0.075 g, 0.75 mmol) at 20° for 360 h. The reaction mixture which became clear after 48 h was checked periodically by TLC (two-dimensional, first direction: mobile phase A; second direction: mobile phase I; standard solutions of III were applied at the start and of I, II, and phthalimide *after* development in H). Two products were detected after 2 days and five products after 15 days, none being identical with I, II, or with phthalimide. About 40 % of the starting material remained unchanged. The reaction mixture was evaporated, freed of triethylamine by several evaporations with benzene and drying *in vacuo*; the residue on PC (in butanol – ethanol – water (4:1:5) to layer) with microbiological development [39] showed no antibiotic activity.

Treatment of methyl 6β-phthalimidopenicillanate (I) with phosphorus oxychloride in dry benzene. Isolation of methyl 6α-phthalimidopenicillanate (II) and 2,2-dimethyl-3(S)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (III). To a previously prepared mixture of pure phosphorus oxychloride (100 ml) and dry benzene (460 ml) was added methyl 6-phthalimidopenicillanate (I; 7.2 g, 0.02 mol). The reaction mixture was heated under reflux for 60 min. Concentration under reduced pressure, followed by repeated evaporation with several portions of benzene, afforded a brown residue, which was dissolved in acetone (250 ml) and treated with charcoal in the cold (1 h). The filtered solution was evaporated with continuous addition of methanol and simultaneous distillation to give a crystalline mass. The crystals were filtered off and washed with cold methanol and finally with a small amount of methylene chloride. The product (3.54 g, 49.2 %) had a m.p. of 217 – 219° and gave no m.p. depression with III prepared from I with triethylamine in methylene chloride (see above). Recrystallization from acetone-dry ether gave pure 2,2-dimethyl-3(S)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (III; 2.95 g, 40.9 %), m.p. 220 – 221°, $[\alpha]_D^{20}$ – 215.4° (c 0.5, methanol); $[\alpha]_D^{20}$ – 202.2° (c 1, pyridine).

The residue (4.45 g) from the combined mother liquors was dissolved in a minimum amount of methylene chloride and chromatographed on a silica gel column (300 g; gradient elution: benzene → mobile phase I; 40 ml). Fractions 24 – 36 yielded a further quantity of III (1.18 g, 16.4 % total yield of III 4.13 g, 57.3 %). On evaporation of fractions 6 – 10, 682 mg material remained which was recrystallized from methylene chloride →–methanol to give 295 mg (4.1 %) of methyl 6α-phthalimidopenicillanate (II), m.p. 181.5 – 183° (lit.[6] 183°) $[\alpha]_D^{20}$ +210.6° (c 1, chloroform). According to mixed m.p., chromatographic behaviour and IR- and NMR-spectra, the compound was identical with the methyl 6α-phthalimidopenicillanate obtained by treatment of methyl 6β-phthalimidopenicillanate (I) with triethylamine (see above). TLC analysis (in I, four consecutive developments): hR_F 47; compared to hR_F 53 for methyl 6β-phthalimidopenicillanate (I).

IR (KBr): 3000w, 1765s, 1740sh, 1720s. NMR (CDCl$_3$): 8.15 (s, CH$_3$), 8.34 (s, CH$_3$), 6.18 (s, COOCH$_3$), 5.34 (s, CH), 460 (d, $J = 2$, CH), 4.42 (d, $J = 2$, CH), 2.16 (phthaloyl).

No compound identical with the starting material, the methyl 6β-phthalimidopenicillanate (I), could be isolated or detected before or after the column chromatography from the mother liquors (over 1 %).

2,2-Dimethyl-3(S)-methoxycarbonyl-6-phenylacetamido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (VI). To a solution of 2,2-dimethyl-3(S)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (III, 360 mg, 1 mmol) in dioxane, (8 ml) hydrazine hydrate (100 mg, 2 mmol) in water (2 ml) was added and the light yellow solution was stored at +4°C for three days. TLC (in G): hR_F 79 (m), 65 (m), 49 (m) and 21 (s; ninhydrin positive). The clear solution was treated simultaneously with 0.5 N phenylacetyl chloride solution in dry acetone (4 ml, 2 mmol) and 1 N potassium hydrogen carbonate (*ca.* 2 ml, 2 mmol) while stirring in an ice-bath at pH 6. TLC (in G): hR_F 80 (w), 72 (s; VI) and 45 (w); (in H): hR_F 52 (w), 25 (w), 13 (s; VI) and 0 (m). The solution was acidified with 2 N hydrogen chloride to pH 2 and evaporated to dryness. The residue was triturated with mobile phase H (10 ml), filtered and washed with H (3 × 5 ml). The combined filtrates concentrated to 5 ml were chromatographed on a silica gel column (35 g in mobile phase

B, elution directly with H, fraction volume 20 ml). Fractions 6 − 10 contained starting material (III, 15 mg, 4.1 %) and fractions 12 − 30 the title compound (VI, 310 mg, 89 %), m.p. 140 − 142°. Recrystallization from dry ethanol gave the pure compound (218 mg, 62.5 %), m.p. 150 − 151°, $[\alpha]_D^{20}$ − 195° (c 1, chloroform).

UV (methanol): 315(9 600), 258(6 400), 204(14 500). IR (KBr): 3180s, 1710s, 1620s, 1550s, 1515s. NMR (C_5D_5N): 8.40, 8.37 (d, 6H, $C(CH_3)_2$), 6.33 (3H, $COOCH_3$), 6.14 (2H, CH_2), 5.40 (d, $J = 5.5$, CH), 2.78 − 2.33 (m, 5H, C_6H_5), 2.06 (d, $J = 8.5$, CH), 0.83 (br, 2H, 2 NH). (Found: C 58.72; H 5.88; N 8.01; O 18.48; S 9.12. Calc. for $C_{17}H_{20}N_2O_4S$ (348.44): C 58.60; H 5.78; N 8.04; O 18.37; S 9.20.)

Sensitivity of VI toward sodium hydroxide in 50 % aqueous ethanol.[13] To a stirred solution of VI (10 mg, 0.0287 mmol) in 50 % aqueous ethanol (10 ml), kept at $25 \pm 0.5°$, 0.5 equiv. of sodium hydroxide (0.15 ml, 0.094 N) was added all at once. The change of pH was followed with a glass-calomel combination electrode (Titrator TTT1c, Radiometer, Copenhagen, Denmark) over a period of 30 min and plotted against time (Fig. 1).

Raney-nickel desulphurization and acid hydrolysis[14] *of the two thiazepine derivatives III and VI.* (a). The phthalimido derivate (III, 360 mg, 1 mmol) was heated under reflux with carefully washed (dry ethanol), pyrophoric Raney-nickel (10 ml) in dry ethanol (35 ml) for 7 h. After removal of the Raney-nickel by filtration (TLC in H: hR_F 62 (w), 55 (s), 36 (w), 30 (m, III) and 12 (w); detection: treatment with iodine vapor) the filtrate was evaporated (260 mg), dissolved in mobile phase H (3 ml) and chromatographed on a silica gel column (35 g in dry mobile phase B: gradient elution: mobile phase $B \rightarrow H$; 450/20 ml). Fractions 16 − 21 contained the main, chromatographically pure product (110 mg) as an oil which could not be brought to crystallize. $[\alpha]_D^{24}$ + 18° (c 1, carbon tetrachloride).

IR (CCl_4): 2920m, 1755m, 1718s, 1700s, 1462m, 1468m, 1390s. NMR (CCl_4): 9.21 (s, 3H), 9.10 (s, 3H), 6.33 (s, 3H), 2.20 (m, 4H), 8.40 − 8.20 (m, 2H), 7.45 − 6.92 (m, 3H), 6.35 − 6.18 (m, 2H).

The compound (40 mg) was heated under reflux in 8 N sulphuric acid (4 ml) for 4 h. After standing in a refrigerator overnight, phthalic acid was isolated (14 mg, 70 %, m.p. 205 − 208°) by filtration. The filtrate was adjusted to pH 6 with barium hydroxide and filtered. The precipitate was washed with hot water, and the filtrate and washings were combined and concentrated *in vacuo* to a volume of 2 ml. This solution was used for TLC.

(b). In the same manner, the phenylacetamido-thiazepine derivative (VI, 70 mg, 0.2 mmol) was desulphurized with Raney-nickel (2 ml) in abs. ethanol (7 ml). The Raney-nickel free filtrate (TLC in H: hR_F 70 (s), 55 (w), 32 (w) and 14 (w); detection: treatment with iodine vapor) was evaporated to dryness, dissolved in 8 N sulphuric acid (4 ml) for 4 h, neutralized to pH 6 with barium hydroxide, filtered, washed, evaporated and dissolved in 2 ml water. This solution was used for TLC.

The results of the TLC analysis, using pre-coated Silica gel G and Cellulose F TLC-plates (Merck) in five various solvent systems are summarized in Table 3. Spots, marked with an asterisk, were very faint and seem to be identical with D-valine (D-, L-, and DL-valine have similar hR_F values in these systems). Detection: ninhydrin spray-reagent (Merck).

Methyl N-[(2'-phenylacetamido-2'-benzylthiocarbonyl)vinyl]2,3-dehydrovalinate (VIII). The reaction was carried out under the same conditions used by Leonard and Ning.[13] A solution of 2,2-dimethyl-3(S)-methoxycarbonyl-6-phenylacetamido-7-oxo-2,3,4,7-tetra-hydro-1,4-thiazepine (VI, 348 mg, 1 mmol) in dry methanol (5.2 ml) was treated at room temperature with 0.2 N methanolic sodium methoxide (5 ml, 1 mmol), followed after 20 min by a solution of benzyl bromide (180 mg, 1.05 mmol) in dry methanol (8.5 ml). After a further 10 min the TLC analysis (in H) showed complete reaction: hR_F 59 (s), 18 (w) and 2 (w); starting material (VI): hR_F 35. The mixture was evaporated to dryness and the organic products were separated from sodium bromide by extraction with chloroform (15 ml). Filtration and evaporation of the chloroform solution followed by recrystallization of the residue from benzene (1.8 ml) gave pure VIII (286 mg, 65.5 %), m.p. 110 − 112°. TLC and TLC* in A: hR_F 26 and 24, resp.; in chloroform: hR_F 19 and 10, resp.

IR (KBr): 3420m, 3340m, 1715s, 1670s, 1655s, 1590s, 1495s. NMR ($CDCl_3$): 8.10, 7.88 (2 s, 2 × 3H, $C(CH_3)_2$), 6.32 (s, 2H, CH_2), 6.23 (3H, $COOCH_3$), 5.87 (s, 2H, CH_2), 2.74 (s, 5H, C_6H_5), 2.66 (s, 5H, C_6H_5), 2.10 − 2.80 (m, 2 − 3H, CH, NH). (Found: C 65.86;

H 5.99; N 6.22; O 14.62; S 7.29. Calc. for $C_{24}H_{26}N_2O_4S$ (438.56): C 65.73; H 5.98; N 6.39; O 14.59; S 7.31.)

On using 1-chloro-2,4-dinitrobenzene instead of benzyl bromide in the above mentioned experiment (*cf.* Leonard and Ning [13]) no reaction was observed on the basis of TLC analysis (in *H*) even after 120 min at room temperature (lit.[13]: after 5 min reaction time 81 % conversion into the corresponding *S*-(2,4-dinitrophenyl)dehydrocysteinyl derivative; see XIII).

Methyl N-[(2'-phthalimido-2'-benzylthiocarbonyl)vinyl]-2,3-dehydrovalinate (XVa). 2,2-Dimethyl-3(*S*)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (III; 2.88 g, 8 mmol) was suspended in dry methanol (450 ml) and treated at room temperature with 0.2 N sodium methoxide solution (40 ml, 8 mmol). After 20 min 0.2 N methanolic benzyl bromide (42 ml, 8.4 mmol) was added in one portion to the bright yellow solution ($[\alpha]_D^{20}$ 0°) and the reaction mixture was kept at room temperature for one h. TLC* analysis (in *A*) showed two main spots: hR_F 47 (m; XVa) and 24 (s; denoted as XVa[i]). The methanolic reaction mixture was divided into two aliquots and worked up in two different ways:

(a). The first aliquot (267 ml) was *evaporated to dryness* and the residue was dissolved in methylene chloride (200 ml) and filtered from the sodium bromide. The TLC* analysis in *A* showed a marked change in the ratio of the two isomers: hR_F 49 (s; XVa) and 23 (m; XVa[i]). The clear solution was evaporated to a small volume (15 ml), treated with dry methanol (30 ml) and evaporated again slowly to a small volume. The separating crystals were filtered off and washed with cold methylene chloride – methanol (1:3). The product (1.52 g, 84.5 %, m.p. 144 – 148°) was recrystallized from methylene chloride → methanol (1.28 g, 71.2 %, m.p. 150 – 152°). According to the TLC* analysis in *A* this product was not entirely pure: hR_F 50 (s; XVa) and 24 (w; XVa[i]), mother liquor: hR_F 50 (w; XVa) and 23 (m; XVa[i]). Chromatographically pure XVa was obtained, when the raw product (1.59 g) was suspended in 0.2 M hydrogen chloride in 75 % dioxane (43 ml). The substance first formed an oil, which dissolved (within 5 – 6 min) and then suddenly crystallised in long needles (945 mg, 59.5 %), m.p. 152.5 – 153°. TLC* in *A*: hR_F 50.

UV (methanol): 312(23 400), 219(46 600). IR (KBr): 3320m, 1760m, 1720s, 1710sh, 1650s, 1585m. (CDCl₃): 1780m, 1725s, 1650m. NMR (CDCl₃): 7.98, 7.87 (2 s, 2 × 3H, C(CH₃)₂), 6.14 (s, 3H, COOCH₃), 5.82 (s, 2H, CH₂), 3.24 (d, *J* = 13, 1H, CH), 2.72 (s, 5H, C₆H₅), 2.15 (m, 4H, phthaloyl), − 0.12 (d, *J* = 13, 1H, NH). (Found: C 63.94; H 4.81; N 6.28; O 17.82; S 7.19; MV (Rast) 465. Calc. for $C_{24}H_{22}N_2O_5S$ (450.53): C 63.98; H 4.92; N 6.22; O 17.76; S 7.12.)

After 4 months storing at room temperature in the dark, the pure compound gave the following TLC* pattern (in *A*): hR_F 61 (w), 50 (s; XVa), 24 (m; XVa[i]), 10 (w) and 0 (w).

The aqueous dioxane-hydrogen chloride mother liquor, containing the two isomers, TLC* in *A*: hR_F 50 (w; XVa) and 24 (m; XVa[i]), was evaporated to dryness and this amorphous residue (580 mg, 36.5 %) was used, parallel with the pure XVa, for acidic hydrolysis in 75 % aqueous dioxane containing 2.0 M hydrogen chlororide/1 at room temperature (see later).

(b). The second aliquot (267 ml) of the methanolic reaction mixture was *concentrated to one third of its volume*, diluted with methylene chloride (400 ml), washed with water (1 × 400 ml, 4 × 150 ml) and dried (MgSO₄). TLC* in *A*: hR_F 47 (m; XVa) and 24 (s; XVa[i]). After evaporation the residue (1.89 g) was chromatographed on a silica gel column (200 g; the column had been prewashed with benzene containing 1 % acetone (200 ml), the residue was applied dissolved in a minimum amount of the same solvent and eluted by gradient techniques: benzene with 1 % acetone → mobile phase *A*: 1000/20 ml). Fractions 45 – 51 contained XVa (420 mg, 23.3 %; TLC* in *A*: hR_F 50), which after recrystallization from methylene chloride → methanol (364 mg, 16.4 %) showed a m.p. of 152 – 153° and by mixed m.p. and spectroscopical evidence was identical with the product obtained by route *a*. The XVa[i] was contained in fractions 59 – 80 (TLC* in *A*: hR_F 24). It was stable in the eluting solvent below + 5° for several days. Evaporation of an aliquot of the solution gave a yellow, non-crystalline, semisolid material, which could not be brought to crystallize. Furthermore all operations even at 20° resulted in a mixture of XVa and its isomer together with decomposition products (strong benzylthiol odour). For IR and NMR analysis two aliquots could, however, be transferred without

isomerisation or decomposition into chloroform and deuteriochloroform solution by careful evaporation in the cold with continuous addition of chloroform and deuteriochloroform, respectively.

IR (CHCl$_3$): 1780w, 1720s, 1665w, 1600w. NMR (CDCl$_3$): 8.02 (s), 7.88 (s), 6.28 (s), 5.86 (s), 2.75 (m), 2.18 (m).

Methyl   N-[(2'-phthalimido-2'-methylthiocarbonyl)vinyl]-2,3-dehydrovalinate   (XVb). This compound was prepared in the same manner as the benzylthiocarbonyl derivative (XVa, route *a*) from III (720 mg, 2 mmol) using methyl iodide as alkylating agent. Recrystallisation from benzene-hexane gave XVb (465 mg, 62.2 %), m.p. 158 − 160°. An analytical sample was obtained by two further recrystallizations from the above solvents, m.p. 160 − 161° (*hR$_F$* values, see Table 4).

IR (KBr): 3390m, 1785m, 1750m, 1718s, 1695sh, 1650s, 1620s, 1595s. NMR (CDCl$_3$): 8.0, 7.96, 7.88, 7.84 (C(CH$_3$)$_2$), 7.76, 7.72 (COOSCH$_3$), 6.25, 6.16 (COOCH$_3$), 3.26 (d, $J = 13$, CH), 2.44 (m, phthaloyl), −0.02 (d, $J = 13$, NH). (Found: C 57.69; H 4.80; N 7.39; O 21.50; S 8.51. Calc. for C$_{18}$H$_{18}$N$_2$O$_5$S (374.43): C 57.74; H 4.85; N 7.48; O 21.37; S 8.56.)

Esterification   of   methyl   N-[(2'-phthalimido-2'-carbothiolic)vinyl]-2,3-dehydrovalinate (XXI) with methanol and subsequent acid hydrolysis in the presence of 2,4-dinitrophenylhydrazine. Isolation of the 2,4-dinitrophenylhydrazones XVI and XXIII. 2,2-Dimethyl-3(S)-methoxycarbonyl-6-phthalimido-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine (III; 720 mg, 2 mmol) was suspended in dry methanol (120 ml) and 0.2 N sodium methoxide (10 ml, 2 mmol) added at room temperature. After 20 min 0.2 N methanolic hydrogen chloride (20 ml, 4 mmol) was added to the bright yellow solution, which was left for 4 days at room temperature and finally heated under reflux for 1 h. TLC* analysis (in *A*): *hR$_F$* 42 (m, elongated), 18 (m, elongated) and 0 (s). After cooling, 0.2 N methanolic (97.5 %) hydrogen chloride solution (400 ml) containing 2,4-dinitrophenylhydrazone (790 mg, 4 mmol) was added. After standing at room temperature for 24 h, the reaction mixture showed the following TLC pattern (in chloroform): *hR$_F$* 51 (s, yellow; XXIII), 39 (s, red), 13 (m, 2,4-dinitrophenylhydrazine) and 0 (w); detection: spraying with 2 N sodium hydroxide. The solution was concentrated to one tenth of its volume (*ca.* 50 ml) and left overnight. After evaporation to dryness the residue (1.15 g; 78 %) was triturated several times with benzene and filtered. The combined filtrates were concentrated to a small volume and chromatographed on a silica gel column (250 g; gradient elution: benzene → mobile phase *D*; 1600/40 ml). Fractions 24 − 31 contained a yellow crystalline substance (338 mg, 62 %), which after recrystallization from methanol melted at 180 − 181° and was by mixed m.p. and IR spectra identical with an authentic sample of 2,4-dinitrophenylhydrazone of methyl 2-oxoisovalerate (see below).

IR (KBr): 3240w, 3160w, 3020w, 1695s, 1625s, 1595s, 1575s, 1525sh, 1510s. (Found: C 46.37; H 4.76; N 17.88; O 30.88. Calc. for C$_{12}$H$_{14}$N$_4$O$_6$ (310.27): C 46.45; H 4.55; N 18.06; O 30.94.)

Evaporation of fractions 63 − 91 containing two substances with very similar chromatographic properties gave a reddish-brown residue (653 mg, 76.5 %). Several recrystallizations from methanol-water containing 1 mmol of hydrogen chloride per 200 mg of substance gave the pure 2,4-dinitrophenylhydrazone of methyl 2-phthalimido-2-formylacetate (XVI), m.p. 210 − 214° (157 mg, 18.4 %).

IR (KBr): 3330m, 3140w, 1760w, 1705s, 1615s, 1590s, 1515s. (Found: C 50.82; H 3.14; N 16.75; O 29.64; S 0.00. Calc. for C$_{18}$H$_{13}$N$_5$O$_8$: C 50.59; H 3.07; N 16.39; O 29.95.)

The product was by mixed m.p., TLC, and IR-spectrum identical to an authentic sample of XVI prepared according to Sheehan and Johnson.[16]

Methyl N-[(2'-phthalimido-2'-carbomethoxy)vinyl]-2,3-dehydrovalinate (XVII). The benzylthiocarbonyl derivative (XVa; 225 mg, 0.5 mmol) was dissolved in dry dioxane (5 ml) and methanol (10 ml), and 0.05 M dry methanolic mercuric acetate (10 ml, 0.5 mmol) was added at room temperature. After 2 h the reaction mixture contained no more starting material according to TLC (*hR$_F$* values, see Table 5). The clear solution was evaporated with continuous addition of benzene to a small volume yielding a white crystalline precipitate (155 mg, 81 %), m.p. 168 − 170° (decomp.), which analysed as benzylthiomercuric acetate.

IR (KBr): 3050w, 1580m-s, 1540m-s, 1500m, 1460m, 1405s. (Found. C 28.31; H 2.76; O 8.39; S 8.31; Hg 52.24. Calc. for C$_9$H$_{10}$O$_2$SHg: C 28.23; H 2.63; O 8.36; S 8.38; Hg 52.40.)

The filtrate was evaporated to dryness and the residue dissolved in benzene and chromatographed on a silica gel column (20 g; gradient elution: benzene →− mobile phase A; 200 ml/20 ml). Fractions 10−17 contained the title compound (XVII; 157 mg, 87 %, m.p. 142−143°), which was recrystallized from methylene chloride −methanol (1:1; 125 mg, 69.5 %), m.p. 143−145° (hR_F values, see Table 4).

IR (KBr): 3360w, 1780m, 1720s, 1680s, 1640s, 1620s, 1455s, 1410s. NMR (CDCl$_3$): 7.98 (s, 3H, CH$_3$), 7.84 (s, 3H, CH$_3$), 6.32 (s, 3H, COOCH$_3$), 6.18 (s, 3H, COOCH$_3$), 3.14 d, $J=13$, 1H, CH), 2.16 (m, 4H, phthaloyl), 0.96 (d, $J=13$, 1H, NH). (Found: C 60.28; H 5.14; N 7.76; O 26.67. Calc. for C$_{18}$H$_{16}$N$_2$O$_6$: C 60.33; H 5.06; N 7.82; O 26.79.)

Hydrolysis of XVII, isolation of O-methyl 2-phthalimido-2-formylacetate (XVIIIb). The carbomethoxy-2,3-dehydrovalinate derivative (XVII; 180 mg, 0.5 mmol) was dissolved and stirred at room temperature in 2 M hydrogen chloride in 75 % aqueous dioxane (5 ml; two phases). After 4.5 h the hydrolysis was complete: TLC analysis (in F): hR_F 48 (w), 33 (s; XVIIIb), 6 (m) and 0 (m): starting material (XVII) hR_F 60; visualisation: 2,4-dinitrophenylhydrazine-potassium ferricyanide reagent.[40] Then the reaction mixture was taken up in methylene chloride (150 ml), washed with water (5 × 40 ml), dried, evaporated to dryness (175 mg) and crystallized from dry benzene (1.1 ml). The O-methyl 2-phthalimido-2-formylacetate (XVIIIb), m.p. 138−140° (79 mg; 64.0 %) was recrystallized from benzene (39 mg; 31.4 %), m.p. 140−142°. The substance was according to mixed m.p., analytical and spectral data identical to a sample of authentic O-methyl 2-phthalimido-2-formylacetate (XVIIIa) prepared according to the literature.[16]

IR (KBr): 3200m, 1782m, 1720s, 1680s, 1650sh, 1625m. NMR (C$_5$D$_5$N): 6.30 (s, 3H, COOCH$_3$), 2.24 (m, 4H, phthaloyl), 193 (1H, CH), −2.97 (1H, COH). (Found: C 58.42; H 3.74; N 5.60; O 32.18. Calc. for C$_{12}$H$_9$NO$_5$: C 58.30; H 3.67; N 5.67; O 32.36.)

XVIIIb gave a 2,4-dinitrophenylhydrazone (XVI), m.p. 214−215.5°, recrystallized from dioxane − water. This substance was identical with the product previously obtained *via* the route III→XIV→XXI→XVII→XVI (see above).

Acidic hydrolysis of XV a and b in the presence of 2,4-dinitrophenylhydrazine. Isolation of the 2,4-dinitrophenylhydrazones XXIV a and b and XXIII. Methyl *N*-[(2'-phthalimido-2'-benzylthiocarbonyl)vinyl]-2,3-dehydrovalinate (XIa; 450 mg, 1 mmol) was dissolved in 0.1 N methanolic (97.5 %) hydrogen chloride solution (400 ml) containing 2 mmol of 2,4-dinitrophenylhydrazine. After standing overnight at room temperature the reaction mixture was evaporated to a volume of 40 ml and left for two days. The separated crystals (625 mg) were filtered off and the mother liquor was concentrated to a small volume and diluted with water to precipitate a further crop of crystals (133 mg) corresponding to a total yield of XXIII and XXIVa of 91.4 %. TLC analysis (in C): hR_F 67 (s, yellow; XXIII) and 47 (s, red; XXIVa); detection: spraying with 2 N sodium hydroxide.

The mixture was chromatographed on a silica gel column (120 g; elution: first 400 ml benzene, then gradient elution: benzene → mobile phase C; 800/40 ml). Fractions 14−19 contained pure XXIII (281 mg, 90.4 %), which was recrystallized from methanol (254 mg, 81.6 %), m.p. 180−181°. This substance was by mixed m.p., IR, and NMR spectra identical with an authentic sample of the 2,4-dinitrophenylhydrazone of methyl 2-oxoisovalerate and with the product previously obtained from XXI.

The second 2,4-dinitrophenylhydrazone was obtained in fractions 44−52 in pure form (451 mg, 87 %). Recrystallization from benzene →− 98 % ethanol containing 1 mmol hydrogen chloride per 250 mg substance gave the pure 2,4-dinitriphenylhydrazone of the *S*-benzyl-2-phthalimido-2-formylthioacetate (XXIVa; 405 mg, 77.8 %) m.p. 110−114° (decomp.).

IR (KBr): 3350s, 3100w, 1775m, 1715s, 1650s, 1615s, 1530s, 1500s. NMR (CD$_3$)$_2$SO: 5.80 (s, CH$_2$), 2.57 (s, C$_6$H$_5$), 2.58 (d, $J=10$, 1H), 1.88 (s, phthaloyl) 1.37 (q, $J=10$, 3, 1H), 1.00 (d, $J=3$, 1H). (Found: C 55.46; H 3.41; N 13.34; O 21.73; S 6.01. Calc. for C$_{24}$H$_{17}$NO$_7$S (519.51): C 55.49; H 3.30; N 13.48; O 21.56; S 6.17.)

The 2,4-dinitrophenylhydrazone of phenyl 2-phthalimido-2-formylacetate [16] (see Scheme 4; XXII), O-benzyl analogue of XXIVa, showed very similar TLC and spectral properties. TLC in D: hR_F 45 (red; XXII) and 47 (red; XXIVa), in chloroform: hR_F 36 (red; XXII) and 37 (red; XXIVa); detection: spraying with 2 N sodium hydroxide.

IR (KBr): 3350m, 3120m, 1750s, 1718s, 1615s, 1540s, 1520s, 1510s. NMR (CD$_3$)$_2$SO: 6.63 (s, CH$_2$), 2.65 (s, C$_6$H$_5$), 2.63 (d, $J=19$, 1H), 2.05 (s, phthaloyl), 1.55 (q, $J=10$, 3 1H), 1.17 (d, $J=3$, 1H), −0.24 (br, NH).

In an analogous experiment methyl *N*-[(2'-phthalimido-2'-methylthiocarbonyl)-

vinyl]-2,3-dehydrovalinate (XVb; 375 mg, 1 mmol) was treated with 2,4-dinitrophenyl-hydrazine in acidic medium. The two 2,4-dinitrophenylhydrazones formed (total 614 mg, 82.5 %; TLC analysis (in C): hR_F 66 (yellow; XXIII) and 43 (red; XXIVb); detection: spraying with 2 N sodium hydroxide) were separated by column chromatography and purified by crystallization as before. In addition to the 2,4-dinitrophenylhydrazone of methyl 2-oxoisovalerate (XXIII; 218 mg, 70.5 %), m.p. 179 − 180°, identical with the previously obtained product, the 2,4-dinitrophenylhydrazone of the S-methyl 2-phthalimido-2-formylthioacetate (XXIVb; 292 mg, 65.8 %) was isolated, which after recrystallization from benzene →— 98 % methanol containing 1 mmol hydrogen chloride per 200 mg of substance had m.p. 112 − 113° (dec.).

IR (KBr): 3340m, 3120w, 1775m, 1715s, 1665m, 1640sh, 1615s, 1585s, 1540sh, 1525s, 1505s. NMR (CD$_3$)$_2$SO: 7.72 (s, CH$_3$), 2.54 (d, $J=10$, 1H), 1.86 (s, phthalimido), 1.37 (q, $J=10, 3$, 1H), 1.00 (d, $J=3$, CH). (Found: C 48.59; H 3.03; N 15.67; O 25.36; S 7.04. Calc. for C$_{18}$H$_{13}$N$_5$O$_7$S (443.41): C 46.76; H 2.95; N 15.79; O 25.26; S 7.23.)

Hydrolysis of XVa and isolation of the S-benzyl 2-phthalimido-2-formylthioacetate (XXa): 2,4-dinitrophenylhydrazone (XXIVa) and diethylacetal (XXVII). Methyl N-[(2′-phthalimido-2′-benzylthiocarbonyl)vinyl]-2,3-dehydrovalinate (XVa; 900 mg) was hydrolyzed in the same manner as XVII for 3.5 h in 2 M hydrogen chloride in 75 % dioxane (20 ml). TLC analysis (in F): hR_F 29 (s, XXa), 6 (m) and 0 (m); the two lower spots disappeared after washing; starting material (XVa): hR_F 59; visualisation: 2,4-dinitrophenylhydrazine-potassium ferricyanide reagent.[40] The crude reaction product (765 mg) was separated from unchanged starting material (53 mg, 5.9 %) by trituration with dry ethanol and the filtered ethanolic solution was evaporated to dryness to give an amorphous residue (XXa; 663 mg), which was used directly for the preparation of XXIVa and XXVII.

Half of the residue (XVa; 330 mg) was converted into the 2,4-dinitrophenylhydrazone in the usual way. Recrystallization from benzene →— 98 % ethanol containing 0.5 mmol hydrogen chloride per 125 mg substance gave the pure 2,4-dinitrophenylhydrazone of S-benzyl 2-phthalimido-2-formylthioacetate (XXIVa; 265 mg, 51.0 %), m.p. 111 − 114° (dec.), by mixed m.p. and IR identical with the compound previously obtained *via* the route XVa→XXIII + XXIVa (see above).

The other half of the residue (XXa; 332 mg) was treated with triethyl orthoformate (163 mg, 1.1 mmol), dry ethanol (0.2 ml, 151 mg, 3.3 mmol) and one crystal of p-toluene-sulfonic acid and stirred at room temperature overnight. Benzene (10 ml) was added to dissolve the crystals formed and the mixture was washed (2 × 2 ml sat. sodium hydrogen carbonate soln., 2 × 2 ml brine) and dried (MgSO$_4$). The TLC analysis (in isopropyl ether) showed a complete reaction: hR_F 39 (s; XXVIII), 17 (w), 13 (w) and 3 (w); starting material (XXa): hR_F 12 (w) and 1 (s). After evaporation of the solvent the residue (295 mg) was chromatographed on a silica gel column (35 g in dry isopropyl ether; elution: isopropyl ether; 20 ml). The fractions 9 − 13 gave after evaporation the S-benzyl 2-phthalimido-2-formylthioacetate diethylacetal (XXVII) as an oil (182 mg; 44.0 %) which rapidly solidified, m.p. 51 − 52°. The product was identical in all respects with an authentic sample (see below).

IR (KBr): 3000m, 2940m, 1765m, 1715s, 1700sh, 1670sh. (Found: C 64.08; H 5.64; N 3.45; O 19.40; S 7.62. Calc. for C$_{22}$H$_{23}$NO$_5$S (413.50): C 63.90; H 5.61; N 3.39; O 19.35; S 7.75.)

2,4-Dinitrophenylhydrazone of methyl 2-oxoisovalerate (XXIII). The 2,4-dinitro-phenylhydrazone of the 2-oxoisovaleric acid was prepared from 2-phenyl-4-isopropyl-ideneoxazolone according to Ramage and Simonsen[41] (m.p. 195 − 196°; lit.[17] m.p. 196 − 197°). Treatment with ethereal diazomethane[42] in dioxane gave the methyl ester XXIII, which was recrystallized from methanol: m.p. 180 − 181° (lit.[17] m.p. 178 − 180°).

IR (KBr): 3240w, 3160w, 3000w, 1695m, 1626s, 1598s, 1580m, 1530sh, 1510s. NMR (C$_6$D$_6$): 8.85 (d, $J=7$, 2CH$_3$), 7.06 (q, $J=7$, CH), 6.55 (s, COOCH$_3$), 2.34 (d, $J=10$, CH), 2.04 (q, $J=10, 3$, CH), 1.06 (d, $J=3$), −4.04 (br, NH).

O-Benzyl 2-phthalimido-2-formylacetate diethylacetal (XXV). O-Benzyl 2-phthalimido-2-formylacetate[16] (XVIIIa; 6.46 g, 0.02 mol) was treated with triethyl orthoformate (3.26 g, 0.022 mol), dry ethanol (4 ml, 3.02 g, 0.066 mol) and p-toluenesulfonic acid (12 mg) and the reaction mixture which first became a clear solution and then deposited crystals was stirred at room temperature overnight. Benzene (100 ml) was added to give a clear solution, which was washed (2 × 40 ml sat. sodium hydrogen carbonate, 2 × 20 ml

brine) and dried (MgSO$_4$). TLC analysis (in isopropyl ether): hR_F 40 (s; XXV), 26 (w), 11 (w) and 0 (w; XVIIIa). After evaporation the residue was recrystallized from methylene chloride →– isopropyl ether giving the pure acetal XXV (6.15 g, 77.5 %) m.p. 72 – 74°. An analytical sample was recrystallized from warm isopropyl ether, m.p. 75 – 76°.

IR (KBr): 2980m, 2940m, 1775m, 1745s, 1718s. NMR (CDCl$_3$): 9.04 (t, J = 7, CH$_3$), 8.82 (t, J = 7, CH$_3$), 6.60 – 6.04 (m, 2 CH$_2$), 4.98 (d, J = 7, CH), 4.80 (s, CH$_2$), 4.52 (d, J = 7, CH), 2.72 (s, C$_6$H$_5$), 2.20 (m, phthaloyl). (Found: C 66.40; H 5.95; N 3.63; O 24.19. Calc. for C$_{22}$H$_{23}$NO$_6$ (397.43): C 66.49; H 5.83; N 3.52; O 24.15.)

This diethylacetal (XXV; 40 mg, 0.1 mmol) was treated at 70° for 2 h with 0.4 N hydrogen chloride in 60 % dioxane (5 ml) containing 0.1 mmol 2,4-dinitrophenyl-hydrazine. TLC analysis (in E): hR_F 48 (w, XXV), 18 (s, red; XXII), 12 (w; DNP) and 0 (w; XVIIIa); detection: spraying with 2 N sodium hydroxide. The reaction mixture was evaporated to a small volume, treated with a few drops of water and kept in the refrigerator to give the 2,4-dinitrophenylhydrazone of O-benzyl 2-phthalimido-2-formyl-acetate (XXII), m.p. 195 – 196°.

IR (KBr): 3340m, 3100m, 1745s, 1715s, 1610s, 1590sh, 1520s, 1505s.

The product was by mixed m.p. and IR-spectra identical with the 2,4-dinitrophenyl-hydrazone prepared directly from XVIIIa.[16]

2-Phthalimido-2-formylacetic acid diethylacetal (XXVI). O-Benzyl 2-phthalimido-2-formylacetate diethylacetal (XXV; 4.8 g, 0.012 mol) was hydrogenated in dry ethanol (90 ml) over palladium charcoal catalyst (0.8 g; Pd cont. 5 %) at room temperature in a Parr hydrogenation apparatus. After 25 min the hydrogenation was complete (0.012 mol of hydrogen taken up) and the catalyst was filtered off. TLC analysis (in isopropyl ether): hR_F 10 (s; XXVI). After evaporation the crystalline residue was triturated with isopropyl ether, filtered and washed to give XXVI (3.65 g; 99 %), m.p. 99 – 101°.

IR (KBr): 2980s, 2920s, 2650w, 2550w, 1765m, 1720s, 1705sh, 1610w, 1470m, 1395s. NMR (CDCl$_3$): 9.0 (t, J = 7, CH$_3$), 8.72 (t, J = 7, CH$_3$), 6.58 – 5.96 (m, 2 CH$_2$), 4.98 (d, J = 7, CH), 4.52 (d, J = 7, CH), 2.20 (m, phthaloyl), – 0.06 (s, COOH). (Found: C 58.59; H 5.75; N 4.59; O 30.96. Calc. for C$_{15}$H$_{17}$NO$_6$ (307.32): C 58.63; H 5.58; N 4.56; O 31.24.)

S-Benzyl 2-phthalimido-2-formylthioacetate diethylacetal (XXVII). (a). 2-Phthali-mido-2-formylacetic acid diethylacetal (XXVI; 3.08 g, 10 mmol) and dry triethylamine (1.01 g, 10 mmol) were dissolved in methylene chloride (15 ml), the solution was cooled to – 10° and ethyl chloroformate (1.08 g, 10 mmol) in methylene chloride (8 ml) was added with rapid stirring. After 10 min benzyl mercaptan (1.24 g, 1 mmol) in methylene chloride (8 ml) was added and the reaction mixture was stirred for 30 min at – 10° and then at 0° overnight. The reaction mixture was diluted with methylene chloride (100 ml), washed (1 × 50 ml brine, 2 × 20 ml sat. sodium hydrogen carbonate, 2 × 20 ml brine) and dried (MgSO$_4$).

TLC analysis (in isopropyl ether): hR_F 62 (w), 41 (s; XXVII), 17 (w) and 8 (w). After evaporation the residue was chromatographed on a silica gel column (35 g in dry isopropyl ether; elution: isopropyl ether; 20 ml). The S-benzyl 2-phthalimido-2-formyl-thioacetate diethylacetal (XXVII) was contained in fractions 23 – 25 and after evapora-tion of the solvent was obtained as an oil (1.06 g, 25.6 %), which slowly crystallized, m.p. 52°.

IR (KBr): 3000m, 2940m, 1765m, 1715s, 1700sh, 1670sh. NMR (CDCl$_3$): 9.04 (t, J = 7, CH$_3$), 8.75 (t, J = 7), 6.64 – 6.00 (m, 2 CH$_2$), 5.85 (s, CH$_2$), 4.92 (d, J = 8, CH), 4.45 (d, J = 8, CH), 2.75 (s, C$_6$H$_5$), 2.20 (m, phthaloyl). (Found: C 64.02; H 5.68; N 3.49; O 19.44; S 7.69. Calc. for C$_{22}$H$_{23}$NO$_5$S (413.50): C 63.90; H 5.61; N 3.39; O 19.35; S 7.75.)

(b). The diethylacetal acid derivative (XXVI; 616 mg, 2 mmol) was refluxed with thionyl chloride (1.2 g, 10 mmol) in dry methylene chloride (10 ml) for 2 h. The reaction mixture was evaporated to dryness, dissolved and evaporated twice with benzene (2 × 20 ml) and finally taken up in dry methylene chloride (10 ml). The chilled solution was added over a 10 min period to a stirred solution of benzyl mercaptan (248 mg, 2 mmol) and triethylamine (222 mg, 2.2 mmol) in dry methylene chloride (3 ml) at 0°. The reac-tion mixture was stirred overnight, treated with methylene chloride (40 ml), and in portions with water (5 ml) containing 3 mmol of sodium hydrogen carbonate at 0°. The organic phase was separated, washed (1 × 5 ml sat. sodium hydrogen carbonate, 2 × 5 ml brine) and dried (MgSO$_4$). TLC analysis (in isopropyl ether): hR_F 60 (w), 42 (s; XXVII), 19 (w) and 9 (w). After evaporation the residue was chromatographed on a silica gel col-umn as described in route a. Fractions 17 – 21 contained the title compound which after

evaporation (272 mg, 32.8 %) gave an oil, which crystallized slowly on standing, m.p. 51 – 52°. According to analytical and spectral data, this substance was identical in all respects with the S-benzyl 2-phthalimido-2-formylthioacetate diethylacetal (XXVII) obtained in a above and furthermore with the compound isolated after degradation of III (via the route III→XVa→XXa→XXVII).

S-*Benzyl 2-phthalimido-2-formylthioacetate 2,4-dinitrophenylhydrazone (XXIVa).* S-Benzyl 2-phthalimido-2-formylthioacetate diethylacetal (XXVII; 413 mg, 1 mmol) was treated at 70° for 2 h with 0.4 N hydrogen chloride in 60 % dioxane (50 ml) containing 1 mmol 2,4-dinitrophenylhydrazine. TLC analysis (in C): hR_F 72 (w; XXVII), 55 (w), 48 (s; XXIVa), 36 (w) and 0 (w); detection: spraying with 2 N sodium hydroxide. Benzene (400 ml) was added and the solution was washed (8 × 20 ml brine), dried (MgSO₄), evaporated to a small volume and chromatographed on a silica gel column (40 g, gradient elution: benzene → mobile phase C; 800/40 ml). Fractions 28 – 31 contained pure XXIVa, which was isolated by evaporation. The residue (310 mg, 59.8 %) was recrystallized from benzene →– 98 % ethanol containing 1 mmol hydrogen chloride per 250 mg substance. The obtained compound (188 mg; 36.2 %) had a m.p. of 111 – 114° and was according to mixed m.p. and IR-spectrum identical in all respects with the 2,4-dinitrophenylhydrazone of S-benzyl 2-phthalimido-2-formylthioacetate (XXIVa) isolated from the acidic hydrolysis of XVa (XVa→XXIII + XXIVa).

IR (KBr): 3360m, 3100w, 1775m, 1718s, 1660s, 1620s, 1590s, 1530s, 1510sh.

The mutual reversible transformations of XVa and XVaⁱ. (a). Pure XVa (100 µg, in methylene chloride) was applied to a Silica gel F₂₅₄ pre-coated plate (Merck) stored at room temperature for 2 h and developed in mobile phase A: hR_F 42 (s; XVa) and 23 (s; XVaⁱ). After 24 h storing hR_F 48 (w), 43 (m; XVa), 24 (s; XVaⁱ) and 0 (w).

A similar experiment with the XVaⁱ (100 µg; see preparation of XVa, route b, fractions 59 – 80) gave after storing for 2 h an identical chromatogram to that obtained for XVa after 24 h storing: hR_F 48 (w), 43 (m; XVa), 24 (s; XVaⁱ and 0 (w)).

The reversibility of this transformation was furthermore demonstrated using two-dimensional, "diagonale"[43] and "direct spot transfer" techniques[44] with similar stationary and mobile phases.

(b). XVa (50 mg, in methylene chloride) was applied with a Desaga "Autoliner" to Silica gel F₂₅₄ pre-coated preparative plates (Merck; 8 plates), stored at room temperature in the dark for 24 h and then developed with chloroform containing 2 % ethanol. The *lower* broader band — corresponding to XVaⁱ — was scraped off, extracted with chloroform, evaporated, dissolved in methylene chloride, applied to preparative Silica gel plates (6 plates) again, stored 4 h at room temperature and developed with chloroform. Two bands appeared again on the chromatogram, now the *upper* band was scraped off, extracted and evaporated. The residue (9.2 mg) was crystallized from benzene-petroleum ether to give a compound, m.p. 148 – 150°, which according to mixed m.p. and IR-data was identical with the starting XVa.

(c). Pure XVaⁱ (about 45 mg; an aliquot from the fractions 59 – 80 obtained in the preparation of XVa, route b) was dissolved in dry methanol (5.5 ml), a catalytic amount of 0.2 N sodium methoxide solution (0.05 ml; 0.01 mmol) was added and the solution was left for 5 h at room temperature. TLC* analysis (in A): hR_F 69 (w), 62 (m), 50 (s; XVa), 24 (s; XVaⁱ) and 0 (w). After evaporation, the residue was chromatographed on a silica gel column (25 g) as in the original experiment (preparation of XVa, route b). From the fractions which contained the XVa (according to TLC* in A) the crystalline compound was isolated (7.8 mg). After recrystallization from methylene chloride →– methanol it had m.p. 149 – 151° and according to mixed m.p. and IR, it was identical with an authentic sample of XVa. The fractions, which contained the unchanged XVaⁱ, were carefully evaporated with simultaneous addition of pure chloroform and the solution, now free of benzene and acetone, was evaporated to give a ca. 5 % solution of XVaⁱ. TLC* of the solution (in A) indicated practically pure XVaⁱ and IR showed the characteristic lines of the compound.

IR (CHCl₃): 3060, 1775w, 1725s, 1665w, 1605w.

(d). A similar experiment to c starting from pure XVa resulted in two main spots on TLC* (in A): hR_F 68 (w), 61 (w), 51 (s; XVa), 24 (s; XVaⁱ) and 0 (w). These experiments proved the reversible mutual transformation of the two isomers (XVa and XVaⁱ) into each other under the catalytic action of an active silica gel surface (a and b) and of sodium methoxide (c and d).

(e). Chromatographically pure XVa and the residue from its mother liquor (see preparation XVa, route *a*), containing a mixture of the two isomers, were in parallel experiments hydrolysed (225 mg each, 0.5 mmol) with 2 M hydrogen chloride in 75 % dioxane (10 ml) as described before. The reactions were checked by TLC* (in *A*), samples being taken every 20 min (see Fig. 2): average hR_F 50 (XVa), 24 (XVa^j), 6 and 0 (hydrolytic products). After 5 h both reaction mixtures were transformed into their 2,4-dinitrophenyl-hydrazones and isolated by column chromatography (with TLC control) as described before. Yields (without recrystallization) of the 2,4-dinitrophenylhydrazone-derivatives of methyl 2-oxoisovalerate and of *S*-benzyl-2-phthalimido-2-formylthioacetate, XXIII and XXIVa, resp., were: (1) from pure XVa, 81.3 % XXIII and 72.5 % XXIVa; and (2) from the mixture of XVa and XVa^j, 76.5 % XXIII and 53.2 % XXIVa.

These experiments showed the existence of the XVa→XVa^j→oxo-compounds pathway on acidic hydrolysis.

REFERENCES

1. Clarke, H. T. and Inouye, J. M. *J. Biol. Chem.* **94** (1932) 541.
2. Stoll, A. and Seebeck, E. *Helv. Chim. Acta* **31** (1948) 189.
3. Wolfe, S., Godfrey, J. C., Holdrege, C. T. and Perron, Y. G. *J. Am. Chem. Soc.* **85** (1963) 643.
4. Morin, R. B., Jackson, B. G., Mueller, R. A., Lavagnino, E. R., Scanlon, W. B. and Andrews, S. L. *J. Am. Chem. Soc.* **91** (1969) 1401.
5. Wolfe, S. and Hasan, S. K. *Chem. Commun.* **1970** 833.
6. Wolfe, S. and Lee, W. S. *Chem. Commun.* **1968** 242.
7. Wolfe, S., Lee, W. S. and Misra, R. *Chem. Commun.* **1970** 1067.
8. Kovacs, Ö. K. J., Ekström, B. and Sjöberg, B. *Tetrahedron Letters* **1969** 1863.
9. Kovacs, Ö. K. J., Ekström, B. and Sjöberg, B. *10th European Peptide Symposium*, Abano-Terme, Italy, September 7 – 13, 1969.
10. Sheehan, J. C. and Cruickshank, P. A. *J. Am. Chem. Soc.* **78** (1956) 3680.
11. Clayton, J. P., Southgate, R., Ramsay, B. G. and Stoodley, R. J. *J. Chem. Soc.* C **1970** 2089.
12. *Private communication*, Beecham Research Laboratories.
13. Leonard, N. J. and Ning, R. Y. *J. Org. Chem.* **32** (1967) 677.
14. Leonard, N. J. and Wilson, G. E. *J. Am. Chem. Soc.* **86** (1964) 5307.
15. Stewart, F. B. and McKinney, P. V. *J. Am. Chem. Soc.* **53** (1931) 1482.
16. Sheehan, J. C. and Johnson, D. A. *J. Am. Chem. Soc.* **76** (1954) 158.
17. Birkinshaw, J. H., Oxford, A. E. and Raistrick, H. *Biochem. J.* **30** (1936) 394.
18. Sheehan, J. C. and Henery-Logan, K. R. *J. Am. Chem. Soc.* **84** (1962) 2983.
19. Houben, J. and Weyl, T. *Methoden der organischen Chemie*, Georg Thieme, Stuttgart 1955, Vol. 9, p. 758.
20. Jackson, J. R. and Stoodley, R. J. *Chem. Commun.* **1970** 14.
21. Ramsay, B. G. and Stoodley, R. J. *Chem. Commun.* **1971** 450.
22. Vlietinck, A., Roets, E., Claes, P. and Vanderhaeghe, H. *Tetrahedron Letters* **1972** 285.
23. Capon, B. and Rees, C. W. *Organic Reaction Mechanisms 1969*, Interscience, London – New York 1960, p. 164.
24. Kwok, W. K., Lee, W. G. and Miller, I. *J. Am. Chem. Soc.* **91** (1969) 468.
25. Lord, E., Naan, M. P. and Hall, C. D. *J. Chem. Soc.* B **1971** 220.
26. Fiandanese, V. Marchese, G. and Maso, F. *Chem. Commun.* **1972** 250.
27. Cram, D. J. *Fundamentals of Carbanion Chemistry*, Academic, New York 1965, Chapter III.
28. Mayers, G. L. and Kovacs, J. *Chem. Commun.* **1970** 1145.
29. Gutowski, G. E. *Tetrahedron Letters* **1970** 1779.
30. Clayton, J. P., Nayler, J. H. C., Southgate, R. and Stove, E. R. *Chem. Commun.* **1969** 129.
31. Jackson, J. R. and Stoodley, R. J. *Chem. Commun.* **1971** 647.

32. Luche, J. L. and Balavoine, G. *Bull. Soc. Chim. France* **1971** 2733.
33. Bose, A. K., Narayanan, C. S. and Manhas, M. S. *Chem. Commun.* **1970** 975.
34. Johnson, D. A., Mania, D., Panetta, C. A. and Silvestri, N. H. *Tetrahedron Letters* **1968** 1903.
35. Hudson, R. F. *Structure and Mechanism in Organo-Phosphorus Chemistry*, Academic, New York 1965, p. 269.
36. Stahl, E. *Chromatographia* **1** (1968) 342.
37. Goldman, M. and Day, R. A. *Ohio J. Sci.* **67** (1967) 190.
38. Wright, D. A. and Wulff, C. A. *J. Org. Chem.* **35** (1970) 4252.
39. Batchelor, F. R., Doyle, F. P., Nayler, J. H. C. and Rolinson, G. N. *Nature* **183** (1959) 257.
40. Stahl, E. *Dünnschicht-Chromatographie*, Springer, Berlin 1967, p. 827.
41. Ramage, G. R. and Simonsen, J. L. *J. Chem. Soc.* **1935** 532.
42. Ronkainen, P. a. *Suomen Kemistilehti* **B 37** (1964) 209; b. *J. Chromatog.* **20** (1965) 403.
43. Stahl, E. *Dünnschicht-Chromatographie*, Springer, Berlin 1967, p. 89.
44. Székely, G. *J. Chromatog.* **42** (1969) 543.