Acta Chemica Scandinavica B 31 (1977) 307—312

Absolute Configurations of Alkylcitric Acids

SVANTE BRANDÄNGE, STAFFAN JOSEPHSON, LARS MÖRCH and STAFFAN VALLÉN

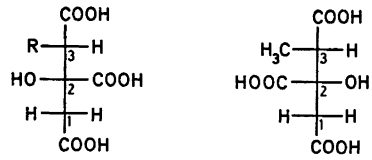
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

The absolute configurations of five naturally occurring alkylcitric acids (*1*—*5*) have been determined. The relative configurations were elucidated by comparison with NMR spectra or GLC retention times of stereospecifically synthesised *R*,R** esters and *R*,S** esters (Fig. 1). The absolute configurations of *1*—*4* (2*S*,3*S*)† and *5* (2*R*,3*S*)† were determined by CD measurements on the natural acids and optically active synthetic acids (*12*—*15*) as their molybdate complexes. Previously reported molecular ellipticities for 2-alkylmalic acids must be corrected.

Five alkylcitric acids (*1*—*5*) have previously been isolated from or detected in natural sources and the determination of their absolute configurations is reported here. The methylcitric acids *1* and *5* are excreted in the urine of humans suffering from propionic acidemia, a rare disease leading to death in infancy.[1] A methylcitric acid has also been found in the culture of *Candida lipolytica* [2] and from the NMR and optical rotation data given [2] we conclude it to be acid *1*. (−)-Decylcitric acid (*2*) is a metabolite produced by a variant of *Penicillium spiculisporum*,[3,4] norcaperatic caid (*3*) is probably responsible for the toxicity of *Cantharellus floccosus*,[5] and the 1-monomethyl ester of the latter (caperatic acid [6]) is a common lichen acid. Agaricic acid (*4*) is a hexadecylcitric acid occurring in *Polyporus officinalis*.[7] Another representative of this structural class, butylcitric acid, has been assumed to be an intermediate in the biosynthesis of the fungal metabolite glauconic acid.[8]

In order to determine the stereostructures of the natural alkylcitric acids we have synthesised such compounds stereospecifically from the epoxides *6* and *7* (Fig. 2) as well as from their racemic modifications. The absolute configurations of *6* and *7* are known [9] since they have been correlated with the known [10–12] lactones of hydroxycitric acids. Evidence for a *trans* opening of the epoxide rings in *6* and *7* on reaction with lithium dialkylcuprate reagents comes from the fact that dimethyl epoxyfumarate, on reaction with lithium dimethylcuprate, gives *erythro*-3-methylmalic acid. Consequently, *6* should yield (2*S*,3*S*)-alkylcitric acids and *7* should yield (2*R*,3*S*)-alkylcitric acids as shown in Fig. 2.

The relative configurations of *2*—*4* were determined by ^{1}H NMR spectroscopy, using the synthetic *R*,R** esters and *R*,S** esters as reference materials; the spectral features used are depicted in Fig. 3. The trimethyl



1: R = CH_3
2: R = $C_{10}H_{21}$
3: R = $C_{14}H_{29}$
4: R = $C_{16}H_{33}$

5

Fig. 1. Absolute configurations of the natural alkylcitric acids. The formulae shown are to be regarded as Fischer projections although the lowest numbered chain members are not placed at the top.

† The numbering is that of 2-hydroxy-1,2,3-alkanetricarboxylic acids.

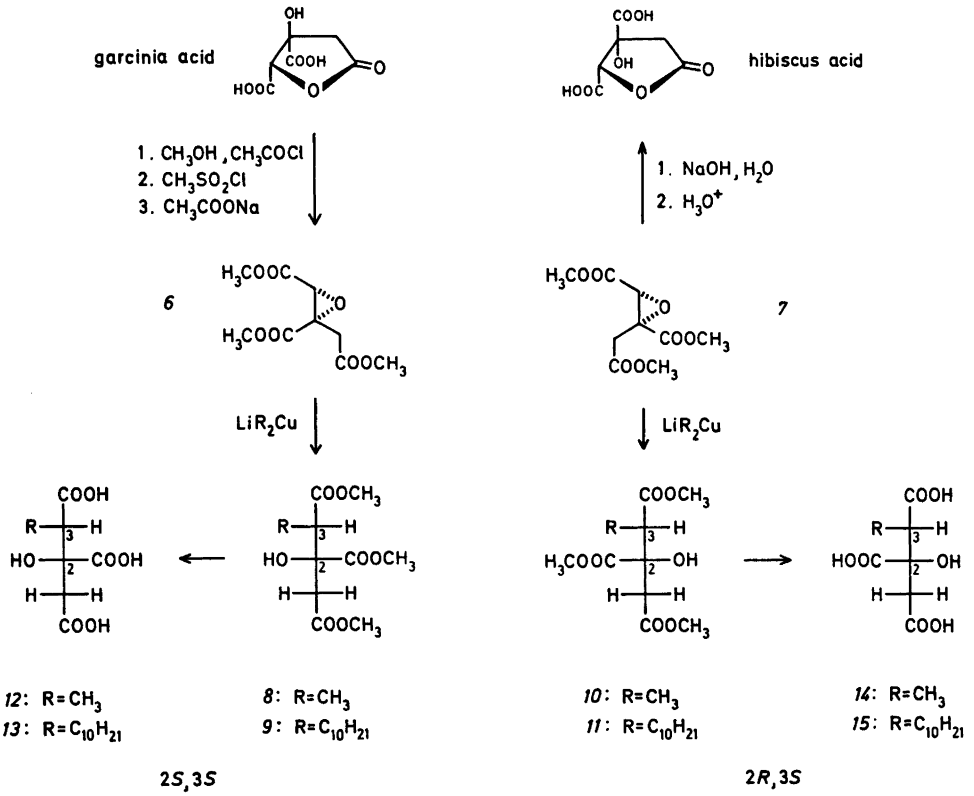


Fig. 2. Synthetic scheme for the correlation of alkylcitric acids with lactones of hydroxycitric acids. The formulae *8* – *15* are to be regarded as Fischer projections although the lowest numbered chain members are not placed at the top.

esters of *2* – *4* gave ^1H NMR spectra containing the wide-spread AB spectrum in Fig. 3a, demonstrating these acids to be either *R,R* or *S,S*. The NMR differences shown in Fig. 3 have been shown to be retained to a large extent in the spectra of the corresponding unesterified acids. The NMR results obtained here allow conclusions to be drawn about the configurations of alkylcitric acids described in the literature. Comparisons of the ^1H NMR data from our methylcitric acids with those given [2] for the methylcitric acid from *Candida lipolytica* indicate that the latter should belong to the *R*,R** pair, although differences in chemical shifts were apparent (see below). This conclusion is supported by comparison of the magnitude of the optical rotations of the trimethyl ester [2] of the *Candida lipolytica* acid with the rotations of *8* and *10* (see below). The NMR chemical shifts published for a crystalline methylcitric acid,[28] synthesised by a Reformatsky reaction,[18] clearly show that this acid is the *R*,S** isomer. The ethylcitric acid which is commercially available should on the same grounds be *R*,S**.[21] It is also possible to distinguish between diastereomeric methylcitric acids by GLC of their trimethyl esters. Using this technique the methylcitric acids obtained from the urine of a patient with propionic acidemia [1] were shown to be present as diastereomers in the approximate ratio 2:1. The retention times of the major and minor peaks were the same as those of the synthetic *R*,R** and *R*,S** isomers respectively.

The absolute configurations of *1* – *5* were studied by CD measurements on the natural and the synthetic acids *12* – *15* as molybdate (VI) complexes.[18] The acids *1* – *4* showed CD

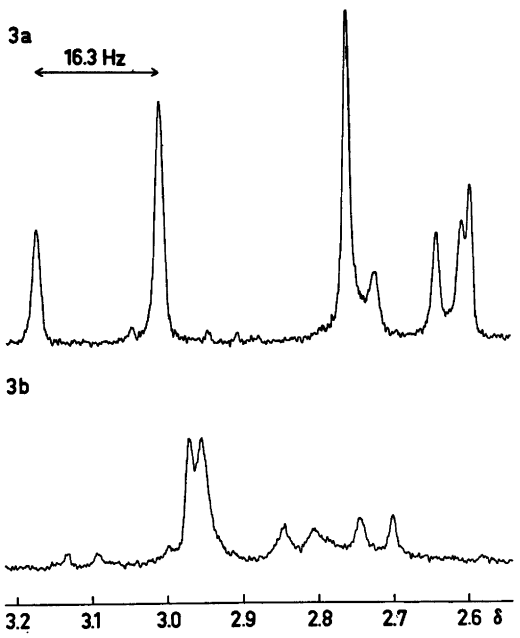


Fig. 3. NMR spectra ($CDCl_3$) of trimethyl esters of diastereomeric decylcitric acids. 3a, *9*; 3b, *11*.

spectra similar to those of *12*–*15*, therefore indicating that *1* is identical to *12*, and *2* is identical to *13*. These acids, as well as *3* and *4*, thus have the *2S,3S* configuration. In a similar investigation, *5* proved to be identical to *14* and is thus *2R,3S*.

Despite the fact that the configurations of *12* and *13* are *2S* and those of *14* and *15* are *2R*, the molybdate complexes of all these acids give CD spectra with similar Cotton effects. A comparison of these spectra with that of (*S*)-malic acid as its molybdate complex [13] indicates that the alkylcitric acids belonging to the *R*,R** pair bond to molybdenum by their carboxyl groups at C-1 and C-2, and that the acids belonging to the *R*,S** pair bond by their C-2 and C-3 groups.

It has been shown that the methyl- and decylcitric acids are biosynthesised from oxaloacetate and propionyl- or lauroyl-CoA.[1,14,15] The *2S* configuration of *1* and *2* requires attack by acyl-CoA at the *si* face of the keto carbonyl group of oxaloacetate; such an attack is analogous to that in the ordinary biosynthesis of citric acid, since the addition of acetyl-CoA to the carbonyl group occurs at its *si* face.[16] The co-occurrence of *1* and *5*, with *5* having the *2R* configuration, is remarkable since the biosynthesis of the latter requires a *re* attack on oxaloacetate. Addition of acetyl-CoA at the *re* face has, however, been found to occur in a few anaerobic bacteria.[17]

Alkylcitric acids have previously been synthesised from diethyl alkyloxaloacetates and Reformatsky reagents in yields ranging from 3 to 15 %.[16–20] By using instead the lithium ester enolate of (−)-menthyl acetate we obtained a 45 % yield of the mixed ester of ethylcitric acid, containing *R*,S** and *R*,R** isomers in the approximate ratio 10:1. In an analogous synthesis, methylcitric acid was obtained in a 30 % yield and only esters belonging to the *R*,S** pair could be detected (GLC, NMR). According to a CD investigation of the ethylcitric acids, the *2S,3R* isomer was formed in approximately 5 % enantiomeric excess.

Correction. The molecular ellipticities of 2-alkylmalic acids reported in two previous papers [22,23] are ten times too large.

EXPERIMENTAL

Epoxide ring opening reactions and isolations of the alkylcitric acids were performed as described previously.[23] The acidic hydrolysis procedure [23] gave a (*2S,3S*)-methylcitric acid which was partly (10 %) isomerized and so an alkaline method was used in this case for preparation of the free acid. The ester was treated with 0.5 M sodium hydroxide solution (25 °C, 4 days) and after washing with ether, the solution was passed through a Dowex 50W-X8 (H^+) column and evaporated to dryness. The crude acid obtained was investigated directly by CD and, after methylation with diazomethane, by GLC. All CD data given below refer to maxima or minima obtained for the alkylcitric acids in aqueous solutions (unless otherwise stated) (pH 3.4 – 3.7) containing molybdate(VI). Further CD conditions have been described previously.[23] Analytical GLC was performed on a Perkin-Elmer 900 instrument equipped usually with a JXR column (3 % on Gas-Chrom Q, 100 – 120 mesh, 0.2 × 180 cm); for the trimethyl methylcitrates an ECNSS-M column (3 % on Chromosorb W, 100 – 120 mesh) was used. Preparative GLC was performed on an Aerograph 1400 instrument using an ECNSS-M column (3 % on Chromosorb W, 100 – 120 mesh, 0.3 × 360 cm) for trimethyl methylcitrates and an SE-30 column (2 % on Chromosorb W, 60 – 80 mesh, 0.3 × 190 cm) for trimethyl decylcitrates. Optical rotations were measured on a Perkin-Elmer 141

polarimeter, NMR spectra were recorded on a Varian XL-100 instrument and mass spectra (GLC-MS) on a Varian MAT 311 spectrometer.

cis-*Epoxide* 6. (±)-*threo*-Trimethyl hydroxycitrate was prepared by osmium tetroxide hydroxylation of *trans*-trimethyl aconitate.[9] Distillation gave the dimethyl ester of (±)-garcinia acid which was then hydrolysed according to Martius *et al.*[24] Resolution of the racemic acid with chinchonine [9] gave, after one recrystallisation, a salt from which garcinia acid could be obtained (85 % optically pure). The *cis*-epoxide *6* was prepared from garcinia acid according to Guthrie *et al.*[9] and it gave $[\alpha]_D^{25}$ −27.9° (*c* 1.0, methanol) showing an 81 % optical purity; lit.[9] value: $[\alpha]_D^{25}$ −34.6° (*c* 1.07, methanol). ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 6 H), 3.72 (s, 3 H), 3.67 (s, 1 H), 3.25, 3.08, 2.83, and 2.66 (AB spectrum, 2 H). MS (*m/e*, relative intensity): M$^+$ − 31 = 201(8), 174(33), 173(46), 145(39), 141(56), 114(45), 113(100), 75(28), 59(87).

trans-*Epoxide* 7. The *trans*-epoxy acid was prepared by epoxidation of *trans*-aconitic acid.[9] Resolution with cinchonidine [9] gave, after one recrystallisation, a salt from which the (+)-epoxy acid could be obtained in 81 % optical purity. It showed $[\alpha]_D^{25}$ +51° (*c* 1.1, water) compared to the lit.[9] value $[\alpha]_D^{25}$ +63.1° (*c* 1.0, water). This acid was esterified with diazomethane to give *7*, $[\alpha]_D^{23}$ +133° (*c* 1.8, chloroform). ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 1 H), 3.81 (s, 3 H), 3.78 (s, 3 H), 3.70 (s, 3 H), 3.11 (s, 2 H). MS (*m/e*, relative intensity): M$^+$ − 31 = 201 (11), 174 (9), 173 (92), 145 (36), 141 (100), 114 (9), 113 (97), 75 (47), 59 (98).

(2S,3S)-Trimethyl methylcitrate (*8*) was obtained in 60 % yield after preparative GLC. $[\alpha]_D^{22}$ +9.7° (*c* 0.3, methanol) for the sample being 81 % optically pure. Reported for the trimethyl ester of the methylcitric acid from *Candida lipolytica*: $[\alpha]_D^{20}$ +12° (*c* 1.5, methanol).[2] MS (*m/e*, relative intensity): M$^+$ = 248 (not observed), 189 (12), 157 (100), 115 (61), 101 (27), 88 (28), 59 (35). ^{1}H NMR ($CDCl_3$): δ 3.98 (s, 1 H), 3.82 (s. 3 H), 3.70 (s, 3 H), 3.67 (s, 3 H), 3.14, 2.98, 2.88 and 2.72 (AB spectrum, 2 H), 2.85 (q, 1 H, J = 7 Hz), 1.20 (d, 3 H, J = 7 Hz). The corresponding *acid 12* showed (nm, $[\theta] \times 10^{-4}$): 275, −0.90; 249, +1.5; 233, −1.1.

(2R,3S)-Trimethyl methylcitrate (*10*) was also obtained in 60 % yield after preparative GLC: $[\alpha]_D^{22}$ −17.7° (*c* 0.75, methanol) for the sample being 81 % optically pure. MS: very similar to that of *8*. ^{1}H NMR ($CDCl_3$): δ 3.98 (s, 1 H), 3.80 (s, 3 H), 3.68 (s, 6 H), 2.95 (q, 1 H, J = 7 Hz), 2.90 (s, 2 H), 1.26 (d, 3 H, J = 7 Hz). The corresponding *acid 14* showed (nm, $[\theta] \times 10^{-4}$): 280, −0.8; 249, +1.5; 233, −0.7; 218, +0.4; 205, −1.1.

(R,R*)-Trimethyl butylcitrate* was purified on a silica gel column with ether − light petroleum (2:3) as eluent. It was necessary to add a little diazomethane solution to the eluate since part of the ester had been hydrolysed on the column. MS (*m/e*, relative intensity): M$^+$ = 290 (not observed), 231 (8), 199 (100), 157 (59), 130 (28), 101 (43), 97 (30), 87 (50). ^{1}H NMR ($CDCl_3$): δ 3.88 (s, 1 H), 3.80 (s, 3 H), 3.70 (s, 3 H), 3.66 (s, 3 H), 3.18 − 2.60 (m, 3 H, see Fig. 3), 1.90 − 0.70 (m, 9 H).

(2R,3S)-Trimethyl butylcitrate: $[\alpha]_{578}^{22}$ −13° (*c* 0.5, chloroform). MS: indistinguishable from that of the *R*,R** isomer. ^{1}H NMR ($CDCl_3$): δ 4.10 (s, 1 H), 3.79 (s, 3 H), 3.70 (s, 3 H), 3.69 (s, 3 H), 3.12 − 2.70 (m, 3 H, see Fig. 3), 1.80 − 0.70 (m, 9 H).

(2S,3S)-Trimethyl decylcitrate (*9*). The crude product was hydrolysed with 2 M sodium hydroxide in aqueous ethanol. After washing with ether the solution was acidified and the acid was extracted into ether. Esterification with diazomethane gave a product approximately 70 % pure (^{1}H NMR) (the presence of contaminating compounds only being revealed by too large a peak from decyl hydrogens). A sample of 90 % purity was obtained after preparative GLC. MS (*m/e*, relative intensity): M$^+$ = 374 (not observed) 315 (5), 283 (100), 241 (23), 214 (17), 163 (25), 101 (46), 87 (60), 74 (27), 69 (24), 59 (27), 55 (36). The ^{1}H NMR spectrum was indistinguishable from that of the trimethyl ester of the natural decylcitric acid,[4] except for the intensity of the signal from the decyl group. The *acid 13* showed (nm, $[\theta] \times 10^{-4}$): 275, −0.8; 250, +1.0; 230, −3.0.

(2R,3S)-Trimethyl decylcitrate (11). MS: indistinguishable from that of *9*. ^{1}H NMR ($CDCl_3$): δ 4.08 (s, 1 H), 3.78 (s, 3 H), 3.70 (s, 3 H), 3.69 (s, 3 H), 3.10 − 2.68 (m, 3 H, see Fig. 3), 1.80 − 0.70 (m, approximately 21 H). The *acid 15* showed in H_2O − THF (1:1) as solvent (nm, $[\theta] \times 10^{-4}$): 277, −0.30; 250, +0.12; 238, −0.32.

Investigation of natural acids. A crude mixture (4.9 mg) of methylcitric acids *1* and *5*, isolated from 40 ml of urine, was supplied by Dr. L. Sweetman (University of California, San Diego, USA). The acids were esterified with diazomethane and the esters were purified by preparative GLC on the ECNSS-M column at 170 °C. Two partially separated peaks were obtained which showed the sample to contain diastereomeric esters in the ratio 1:2, (retention times 14.8 and 16.4 min, respectively). The first eluted component (*R,S* or *S,R*) was obtained in more than 95 % purity and the second (*R,R* or *S,S*) in approximately 95 % purity. Found for *1* (nm, $[\theta] \times 10^{-4}$): 274, −1.1; 249, +1.5; 232, −1.6; 217, +1.4. Found for *5* (nm, $[\theta] \times 10^{-4}$): 280, −0.7; 249, +1.5; 232, −0.7; 222, +0.2; 207, −0.9. From the gas chromatographically determined relative configurations and these CD data it follows that *1* is identical to *12* and that *5* is identical to *14*.

The methylcitric acid from *Candida lipolytica* showed [2] in D_2O δ 1.17 (3 H, d), 2.61 (1 H, q),

2.73 (2 H, q, $J = 15.6$ Hz). Our acid *12* showed (in D_2O with sodium 3-(trimethylsilyl)propanesulfonate as internal reference): δ 1.20 (3 H, d), 2.90 (1 H, q), 2.91 and 3.17 (AB spectrum, 2 H, $J = 16$ Hz), whereas *14* showed δ 1.25 (3 H, d), ≈ 2.98 (1 H, obscured by the AB spectrum), 2.95 and 3.01 (AB spectrum, 2 H, $J = 16$ Hz). The spectra of these acids in alkaline D_2O solution did not show greater resemblance to that of the natural product than did the spectra of the acids. In our opinion, however, only the AB spectrum from *12* but not that of *14* resembles a 1:3:3:1 quartet. The specific rotation of the trimethyl ester of the natural acid is $[\alpha]_D^{20}$ +12° (*c* 1.5, methanol) and this value is in good agreement with that obtained for *8* (being 81 % optically pure): +9.7°. It differs clearly from that obtained for *10* (also 81 % optically pure): −17.7°.

(−)-*Decylcitric acid* [3,4] showed (nm, $[\theta] \times 10^{-4}$): 273, −2.4; 250, +2.3; 234, −3.2; 217, +3.0. With H_2O-THF (1:1) as solvent values of 285, −0.2; 249, +1.1; 225, −1.3; 210, +0.7 were obtained.

Norcaperatic acid (*3*),[5,25] obtained by hydrolysis of caperatic acid,[6] showed in H_2O-THF (1:1) (nm, $[\theta] \times 10^{-4}$): 282, −0.4; 247, +0.6; 225, −0.7.

Agaricic acid (*4*) was purchased from E. Merck and was recrystallised from ethanol. It showed in H_2O-THF (1:1) (nm, $[\theta] \times 10^{-4}$): 284, −0.3; 249, +1.2; 227, −1.5; 210, +0.8.

Trimethyl ethylcitrate was synthesised from diethyl ethyloxaloacetate and the lithium ester enolate of (−)-menthyl acetate in analogy with previously performed condensations with (−)-menthyl acetate.[22] Diethyl (−)-menthyl ethylcitrate was obtained in 45 % yield (NMR, piperonal as internal standard). Hydrolysis and subsequent methylation with diazomethane gave trimethyl ethylcitrate containing (*RS,SR*) and (*RR,SS*) isomers in the approximate ratio 10:1 (GLC, NMR). An analytical sample of the same composition was obtained by preparative GLC and showed $[\alpha]_{578}^{20}$ +2.1° (*c* 2.7, chloroform). $M^+ = 262$ (not observed), 203 (11), 171 (100), 129 (75), 112 (30), 111 (33), 87 (28), 59 (36). CD values obtained (nm, $[\theta] \times 10^{-4}$): 267, +0.2; 249, −0.4; 232, +0.2. These values show that the *2S,3R* isomer predominates over its enantiomer.

Dimethyl erythro-*3-methylmalate*. Dimethyl *trans*-2,3-epoxybutanedioate was prepared from the corresponding diacid [26] and diazomethane. It was then treated with lithium dimethylcuprate (as described for *6* and *7*), giving a product indistinguishable (GLC-MS) from an authentic sample of dimethyl *erythro*-3-methylmalate. The latter was obtained from the bis(*p*-bromophenacyl) ester [27] by alkaline hydrolysis, acidification and reaction with diazomethane. The *erythro* and *threo* isomers were well separated on the ECNSS-M GLC column.

Acknowledgements. We cordially thank Dr. L. Sweetman for supplying the methylcitric acids, Dr. A. Måhlén for a sample of decylcitric acid, Dr. G. Aulin-Erdtman for a sample of the bis(*p*-bromophenacyl) ester of *erythro*-3-methylmalic acid and Professor S. Gronowitz, Kemicentrum, Lund, for the CD spectropolarimetry facilities placed at our disposal. Mr. Jan Glans and Dr. Rolf Håkansson kindly measured the CD spectra, Mr. Bengt Lindqvist the NMR spectra, and Mrs. Lena Liedgren and Mr. Frank Lindh the mass spectra. This work has been supported by the Swedish Natural Science Research Council.

REFERENCES

1. Ando, T., Rasmussen, K., Wright, J. M. and Nyhan, W. L. *J. Biol. Chem. 247* (1972) 2200.
2. Tabuchi, T., Serizawa, N. and Ohmomo, S. *Agr. Biol. Chem. 38* (1974) 2565.
3. Gatenbeck, S. and Måhlén, A. *Acta Chem. Scand. 22* (1968) 2613.
4. Brandänge, S., Josephson, S., Måhlén, A., Mörch, L. and Vallén, S. *Acta Chem. Scand. B 30* (1976) 177.
5. Miyata, J. T., Tyler, V. E., Jr., Brady, L. R. and Malone, M. H. *Lloydia 29* (1966) 43.
6. Brandänge, S., Mörch, L. and Vallén, S. *Acta Chem. Scand. B 29* (1975) 889, and references therein.
7. Thoms, H. and Vogelsang, J. *Justus Liebigs Ann. Chem. 357* (1907) 145.
8. Bloomer, J. L., Moppett, C. E. and Sutherland, J. K. *Chem. Commun.* (1965) 619.
9. Guthrie, R. W., Hamilton, J. G., Kierstead, R. W., Miller, O. N. and Sullivan, A. C. *Ger. Offen.* 2,258,955 (1973); *Chem. Abstr. 79* (1973) 78134.
10. Boll, P. M., Sørensen, E. and Balieu, E. *Acta Chem. Scand. 23* (1969) 286.
11. Glusker, J. P., Minkin, J. A. and Casciato, C. A. *Acta Crystallogr. B 27* (1971) 1284.
12. Glusker, J. P., Minkin, J. A. and Soule, F. B. *Acta Crystallogr. B 28* (1972) 2499.
13. Voelter, W., Bayer, E., Barth, G., Bunnenberg, E. and Djerassi, C. *Chem. Ber. 102* (1969) 2003.
14. Måhlén, A. *Eur. J. Biochem. 22* (1971) 104.
15. Tabuchi, T. and Uchiyama, H. *Agr. Biol. Chem. 39* (1975) 2035.
16. Hanson, K. R. and Rose, I. A. *Proc. Nat. Acad. Sci. U.S.A. 50* (1963) 981.
17. Gottschalk, G. *Eur. J. Biochem. 5* (1968) 346, and previous papers.
18. Habicht, E. and Schneeberger, P. *Helv. Chim. Acta 39* (1956) 1316.
19. Graf, E. and Liu, K.-C. *Arch. Pharm. 306* (1973) 366.
20. Asano, M., Arata, Y. and Matsui, K. *J. Pharm. Soc. Jpn. 75* (1955) 1568.

21. Robinson, B. H., Williams, G. R., Halperin, M. L. and Leznoff, C. C. *Eur. J. Biochem. 15* (1970) 263.
22. Brandänge, S., Josephson, S. and Vallén, S. *Acta Chem. Scand. 27* (1973) 3668.
23. Brandänge, S., Josephson, S. and Vallén, S. *Acta Chem. Scand. B 28* (1974) 153.
24. Martius, C. and Maué, R. *Hoppe-Seyler's Z. Physiol. Chem. 269* (1941) 33.
25. Asano, M. and Ohta, Z. *Ber. Dtsch. Chem. Ges. 66* (1933) 1020.
26. Payne, G. B. and Williams, P. H. *J. Org. Chem. 24* (1959) 54.
27. Aulin-Erdtman, G., Tomita, Y. and Forsén, S. *Acta Chem. Scand. 17* (1963) 535.
28. Cheema-Dhadli, S., Leznoff, C. C. and Halperin, M. L. *Pediat. Res. 9* (1975) 905.

Received October 25, 1976.