exchange matrix. The nature of this reaction was not established, but it was found that the sulfhydryl reagents *N*-ethylmaleimide and $HgCl_2$ did not prevent the decomposition. However, only a minor portion of the total radioactivity was released in the first 5 ml of effluent, which contained the radioactive MEG liberated from labeled albumin by treatment with thiols. This portion could be accounted for in the measurement by control experiments, and the accuracy was therefore sufficient to allow quantitative determinations of the liberation of MEG.

To demonstrate the utility of the technique the reaction of thiols and albumin labeled with [^{14}C]-AET was studied. Treatment of 0.075 μmol labeled albumin with 0.2 μmol of unlabeled AET (which isomerizes to MEG at pH-values > 7) at pH 7.5, liberated 75 % of the radioactivity within the shortest possible time of measurement. To investigate the possibility to follow the kinetics, the splitting reaction was run at lower pH values. In these experiments penicillamine, mercaptoethylamine, or glutathione were used. Fig. 1

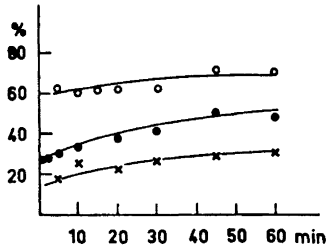

Fig. 1. Release of radioactivity (per cent of total activity) from [^{14}C]-AET-labeled albumin by treatment with mercaptoethylamine or glutathione at pH 5 for different periods of time. ○, 0.063 μmol albumin and 0.2 μmol GSH; ●, 0.063 μmol albumin and 0.2 μmol MEA; ×, 0.063 μmol albumin and 0.066 μmol MEA.

demonstrates that even at pH 5 a substantial amount of radioactivity is released almost instantaneously, and that glutathione is about twice as active as mercaptoethylamine. It should also be noted that in no case more than 85 % of the radioactivity was released from albumin. Whether this means that part of the label is inaccessible to the reagents used or that the label is bound covalently not exclusively by disulfide linkage has not been established.

Similar experiments have been carried out with [^{14}C]-labeled insulin and hemoglobin. In the latter case CM-Sephadex was substituted for DEAE-cellulose in the chromatographic columns. It was found that the radioactivity was much less easily removed from hemoglobin than from albumin by treatment with glutathione.

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Asymmetric Synthesis of (+)-Diethyl Citramalate

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Two hydroxyacids, 2-isobutylmalic acid [1] and 2-benzylmalic acid,[2] have been found in optically active forms as components in *Orchidaceae* alkaloids. (+)-2-Methylmalic acid, (+)-citramalic acid, is known to have the (*S*)-configuration.[3] Asymmetric syn-

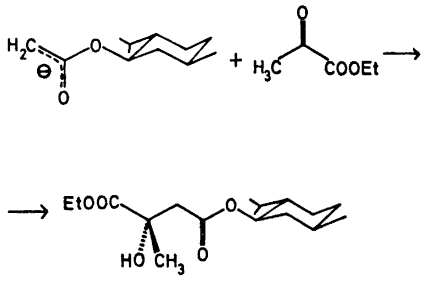
thesis of diethyl citramalate has therefore been investigated in order to determine its steric course and find suitable conditions for similar syntheses of the two new acids.

In a high optical yield (93 %) synthesis of (*S*)-(+)-3-hydroxy-3-phenylbutyric acid a solution of diethylaminomagnesium bromide was added to a mixture of acetophenone and (−)-menthyl acetate.[4,5] We synthesised ethyl (−)-menthyl citramalate (I) from ethyl pyruvate, (−)-menthyl acetate and diethylaminomagnesium bromide, using an addition order different from that above. The product I was then converted into diethyl citramalate (II). As the yield and optical yield in this synthesis were moderate, other bases were tested, including lithium bis(trimethylsilyl)-amide [6] and lithium diisopropylamide.[7] The yields, estimated by NMR and GLC, and the optical yields, calculated from the optical rotations of II and of authentic (*S*)-(+)-diethyl citramalate, are given in Table 1. When diisopropylaminomagnesium bromide or isopropylmagnesium chloride [8] were used as bases the yields of I were negligible and poor (2 %), respectively.

Table 1. Yields and optical yields in asymmetric syntheses of diethyl citramalate.

Base	Yield of I %	$[\alpha]_D$ of I	$[\alpha]_D$ of II	Optical yield %
Et_2NMgBr	20	−56°	+3.7°	19
$(Me_3Si)_2NLi$	60	−48°	+4.7°	24
$(Me_2CH\text{-})_2NLi$	75	−48°	+5.1°	26

The reaction between ethyl pyruvate and the Grignard reagent from (−)-menthyl bromoacetate (THF, 20°, 15 h) gave a low yield of I (10 %), and also a low optical yield (10 %) of (+)-II. Similar reactions, using the organozinc compound, have previously been used in asymmetric synthesis of hydroxyacids.[9,10]



I, predominant stereoisomer

Experimental. Analytical GLC was carried out on a JXR column (3 % on Gas-Chrom Q, 100−120 mesh, 0.2×180 cm) using a Perkin-Elmer 900 chromatograph. Preparative GLC was carried out on an SE-52 column (15 % on Chromosorb AW DMCS, 60−80 mesh, 0.8×155 cm) at 180° for compound I and at 100° for compound II. These separations were performed on an Aerograph A-90-P instrument. Yields of I are estimated from GLC and NMR spectra of crude reaction mixtures. Optical rotations were measured on a Perkin-Elmer 141 polarimeter.

(S)-(+)-*Diethyl citramalate* was prepared by refluxing an ethanol solution of the acid [11] and conc. sulphuric acid for 30 h. The ester, after purification by preparative GLC, showed $[\alpha]_D^{24}$ +19.4° (*c* 2.2, chloroform).

Asymmetric synthesis of I. (a) To a solution of diethylaminomagnesium bromide (prepared from 63 mmol of starting materials) in ether (50 ml), kept at 0°, was first added a solution of (−)-menthyl acetate (4.0 g, 21 mmol) in toluene (15 ml), followed by a solution of ethyl pyruvate (2.4 g, 21 mmol) in toluene (10 ml). The reaction mixture was stirred at 0° for 2 h, and was then poured into cold dilute sulphuric acid. The organic layer was dried (Na_2SO_4), volatile components distilled off (1 mm, 100°), and the product I was isolated by preparative GLC.

(b) A solution of butyllithium in ether (5 ml, 1.5 M) was added under stirring (N_2) to a solution of hexamethyldisilazan (1.25 g, 7.6 mmol) in tetrahydrofuran (20 ml). After reflux (30 min) the solution was kept cool (−70° to −80°) during the addition of a solution of (−)-menthyl acetate (1.40 g, 7.35 mmol) in tetrahydrofuran (10 ml). After stirring for 30 min, a solution of ethyl pyruvate (1.0 g, 8.6 mmol) in tetrahydrofuran (5 ml) was added, and the temperature was then allowed to reach 0°. The reaction mixture was worked up as above yielding I. (Found: C 65.1; H 9.73; O 25.3. Calc. for $C_{17}H_{30}O_5$: C 64.9; H 9.62; O 25.4.)

(c) To a solution of lithium diisopropylamide [7] (30 mmol) in ether (20 ml) and tetrahydrofuran (30 ml), kept cooled (−60° to −70°), was added a solution of (−)-menthyl acetate (4.9 g, 26 mmol) in tetrahydrofuran (20 ml). After 1.5 h, ethyl pyruvate (4.0 g, 34 mmol) was added, and after further 40 min

the temperature was allowed to reach 0° and the reaction mixture was worked up as above.

Diethyl citramalate (II) was obtained from crude I by treatment with ethanol and conc. sulphuric acid (reflux for 3 weeks), followed by preparative GLC. Another method, giving similar results, was also used. The crude reaction product was hydrolysed by treatment with potassium hydroxide (2 M) in ethanol (reflux overnight). Water was added, and the mixture was washed several times with ether. The aqueous layer was acidified, the solvent evaporated, and the residue was treated with ethanol and conc. sulphuric acid (reflux 3 days).

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An X-Ray Single Crystal Study of $K_2HgCl_4.H_2O$

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In connection with studies on the crystal chemistry of inorganic compounds of mercury(II) in different environments, the present X-ray single crystal investigation of $K_2HgCl_4.H_2O$ was performed. The compound has earlier been the object of X-ray diffraction studies.[1,2] The position of all non-hydrogen atoms were derived from intensity data and geometrical considerations in the study in 1938.[1] In the next work on the structure in 1955,[2] the positions of the mercury, potassium, and chlorine atoms were determined from two-dimensional Fourier projections.[2] The positions of the oxygen and hydrogen atoms were not found, however.

Considering the state of the experimental X-ray technique and of the numerical calculation methods at the time for the earlier investigations, the accuracy in the determination of the positions of the light atoms could not be particularly high because of the presence of the heavy mercury atoms. Our aim was to redetermine the positions of the light atoms in order to discuss the coordination of the mercury atoms. The compound $K_2HgCl_4.H_2O$ crystallizes in the orthorhombic space group *Pbam* (No. 55) with four formula units in a unit cell with the dimensions $a = 8.258$ Å, $b = 11.662$ Å, $c = 8.925$ Å, and $V = 860$ Å^3. X-Ray single crystal diffractometer data (PAILRED) were collected using MoK-radiation and a graphite monochromator. A suitable needle-shaped crystal was rotated along [001] and the recorded data resulted in 843 independent reflections with intensities larger than $3\sigma_I$. The intensities were corrected for absorption; the linear absorption coefficient was 203 cm^{-1}. The positions of the mercury atoms were obtained from three-dimensional Patterson functions and the positions of the chlorine, potassium and oxygen atoms from difference Fourier syntheses. A least-squares refinement of the positional parameters of all non-hydrogen atoms including one scale factor was at first performed with isotropic tem-