of these two acids. Commercial ethyl stearate contained free stearic acid. The presence of methyl esters could be shown in supposedly pure acids. Unsaturated triglycerides prepared according to accepted procedures[11] and having correct iodine values contained triglyceride, diglyceride, monoglyceride and methyl ester. Monoglycerides distilled in small lots in high vacuum contained free acid. These examples establish the method as useful for proving the identity and purity of lipids without appreciable loss of material.

Experimental

Paper.—Whatman No. 1 was cut in strips 12.5 cm. wide and 44 cm. long across the fiber and dried for 1 hour at about 200°. They were drawn through a solution of 5% silicone in ether (Dow Corning 200 fluid, viscosity 10 cs.), air-dried, and stored in a desiccator over calcium chloride. After such treatment, the paper is only slightly water repellent.

Lipids.—The amounts of lipid applied to the paper should be adjusted to the particular problem. Calibrated capillaries were used for measuring the desired amount of lipid solution in ether. It is advisable to start with small amounts (20 γ) which reveal the major components. In subsequent runs, higher amounts (up to 500 γ) should be used. Spots of major components with similar R_f values will then overlap but minor components having sufficiently different R_f values are easily detected.

Experimental Conditions.—The chromatograms were developed in ascending technique at approximately 20° within 16 hours to a height of about 20 cm. The paper strips were suspended by threads inside Pyrex glass cylinders (15.5 × 46 cm.). Modeling clay served for sealing glass plates to the tops of the chambers.

Indicator Methods

(a) **α-Dextrin–Iodine.**—α-Dextrin was prepared in this Laboratory.[12] The chromatograms were sprayed with a solution of 1% α-dextrin in 30% ethanol and air-dried. They were then placed into a humidifying chamber at room temperature for 1 hour. Humidity facilitates the subsequent iodine reaction which was carried out with iodine vapors in a dry container. Spots of saturated alcohols, fatty acids, esters and monoglycerides remain white, whereas the corresponding unsaturated compounds, which were white in the beginning, turn yellow or even brown after prolonged treatment. The background is violet due to the α-dextrin–iodine complex. The color fades within a few days but can be brightened up again by repeating the iodine treatment. Amounts of about 20 γ of acids or monoglycerides can be demonstrated on actual chromatograms by this technique.

(b) **Pancreatin.**—Di- and triglycerides are accessible to procedure a when split enzymatically into free acids and monoglycerides. The chromatograms were sprayed with a solution of 1% pancreatin (Takamine Labs., Clifton, N. J.) in water and incubated for about 24 hours at 37° in a moisture chamber. After air-drying they were subjected to the above α-dextrin–iodine technique. About 200 γ of a saturated triglyceride can be detected.

(c) **Iodine.**—Unsaturated alcohols, acids, esters and glycerides can be detected by exposure to iodine vapors. The spots are deep yellow or brown on a white background. Less than 20 γ of unsaturated material can easily be detected in the chromatogram.

(d) **Lead Tetraacetate.**—The air-dried paper strips were sprayed with a solution of 1% lead tetraacetate in absolute benzene. White spots on a brown background of lead dioxide are characteristic for monoglycerides or free glycerol. The sensitivity is 50 to 100 γ of monoglyceride in actual chromatograms.

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Preparation of β-Methylglutamic Acid[1]

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β-Methylglutamic acid was desired as a metabolite antagonist, and its synthesis was, therefore, undertaken. This compound has been prepared by Smrt and Sorm,[2] by a Schmidt reaction, and the α- and γ-methyl isomers were also obtained. The latter two amino acids had previously been synthesized by Gal, Avakian and Martin, and by Fillman and Albertson, respectively.[3,4]

It seemed logical to suppose that the β-methyl isomer might be available by an extension of the glutamic acid synthesis of Snyder, Shekelton and Lewis,[5] involving the condensation of ethyl acetamidomalonate with methyl acrylate. The reaction between ethyl acetamidomalonate and ethyl crotonate was studied, and the intermediate addition product was isolated as a solid of m.p. 73.5–74.5°. Contrary to the results obtained in the case of the γ-methyl isomer, loss of a carbethoxy group was not observed.

The intermediate was hydrolyzed and decarboxylated by heating with hydrochloric acid, furnishing a solution from which *dl*-β-methylglutamic acid could be obtained A second *dl*-form of the amino acid may be present in the mother liquors but it was not observed. The β-methylglutamic acid gave an N-benzoyl derivative which crystallized from water as a monohydrate.

In the corresponding synthesis of glutamic acid from methyl acrylate, previous workers did not isolate the addition product, but hydrolyzed the reaction mixture directly. This preparation was repeated, using ethyl acrylate, and the intermediate isolated and found to be N-acetyl-α-carbethoxyglutamic acid diethyl ester, a solid of m.p. 81–82.5°.

Experimental

N-Acetyl-α-carbethoxy-β-methylglutamic Acid Diethyl Ester.—A solution of 21.7 g. (0.1 mole) of ethyl acetamidomalonate in 150 ml. of absolute ethanol was treated with a solution of 400 mg. of sodium in 10 ml. of absolute ethanol. This mixture was treated slowly with 18.7 ml. (0.15 mole) of ethyl crotonate, and then refluxed 9 hours and left overnight. To the solution was added 5 ml. of acetic acid and 20 ml. of water, and most of the solvent removed by vacuum distillation.

The residue was steam distilled under water-pump vacuum for one hour, leaving a light brown oil in a colorless solution. After ice-cooling for one hour, crystals formed, and soon all of the oil had solidified. The crystals were filtered, washed with water, and dried and weighed 17.72 g. The filtrates, on concentrating and seeding, gave a second crop of 2.29 g. The total yield was 20.01 g. (60.5%) though more was still present in the filtrates. This compound was recrystallized several times from water, and then had m.p. 73.5–74.5°.

Anal. Calcd. for $C_{15}H_{25}NO_7$: C, 54.38; H, 7.55. Found: (I): C, 54.03; H, 7.02. Found (II): C, 54.25; H, 7.03.

β-Methylglutamic Acid.—For hydrolysis, 5 g. of the ester was refluxed overnight with 50 ml. of concentrated hydro-

(1) The work described in this paper was aided by a grant to Prof. D. M. Greenberg from the National Cancer Institute, United States Public Health Service.

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chloric acid, and the solution was then evaporated to dryness *in vacuo*. Water was added to the residue, and the evaporation repeated, this operation being done several times. The glue-like mass was treated with a minimum of warm water for solution (5 ml.). It was found that the amino acid could best be isolated from this solution by titration with lithium hydroxide, and making use of the solubility of lithium chloride in alcohol. The solution was treated dropwise with concentrated lithium hydroxide solution as long as a test portion did not give a precipitate with several volumes of ethanol (*p*H 2.5–3). Ten volumes of ethanol then were added slowly during 20 minutes, and the liquid filtered and left to stand, when the amino acid gradually separated as crystalline grains. After five hours, the product was filtered, washed with ethanol and dried (weight 1.65 g.). The filtrates, on standing overnight, gave an additional 150 mg. The total yield was 1.80 g. or 74%. This was the best yield obtained in several runs, the difficulty being in adjusting the amount of lithium hydroxide used. If too much of the alkali is employed, an amorphous high-melting product precipitates before the amino acid separates, while if too little lithium hydroxide is added, some of the amino acid remains in solution after alcohol is added. The amorphous product may be the monolithium salt of the β-methylglutamic acid.

The methylglutamic acid was recrystallized by adding 3–4 volumes of alcohol and seed to its concentrated aqueous solution. The highest m.p. observed, after several recrystallizations, was 169.5–170.5°.

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.72; H, 6.83. Found: C, 44.58; H, 6.72.

Both the amino acid and the high-melting substance gave purple colors on warming with a dilute aqueous acetic acid solution of ninhydrin.

N-Benzoyl-β-methylglutamic Acid Monohydrate.—The amino acid was benzoylated by the procedure of Bullerwell, Lawson and Morley,[6] as used for glutamic acid, giving a white, crystalline, hydrated, benzoyl derivative. This compound was recrystallized several times from water for analysis. On heating, it melted at 114–116°, with partial solidification above 120°, forming the anhydrous acid which melted about 142°.

Anal. Calcd. for $C_{13}H_{17}NO_6$: C, 55.12; H, 6.01. Found: C, 54.82; H, 5.83.

N-Acetyl-α-carbethoxyglutamic Acid Diethyl Ester.—The addition of ethyl acrylate to ethyl acetamidomalonate was carried out in a manner similar to that used for the crotonate. The reactants were mixed more slowly and the product was extracted by ether after the steam distillation. Evaporation of the ether solution left a pasty solid; 43.4 g. (0.2 mole) of the acetamidomalonate gave 51 g. of product or 80.4% yield. This ester was best recrystallized from ether–petroleum ether (2:1). After three recrystallizations, it had m.p. 81–82.5°. The analysis indicated that a carbethoxy group was not lost in the condensation.

Anal. Calcd. for $C_{14}H_{23}NO_7$; C, 52.997; H, 7.26. Found: 52.67; H, 6.77. Calcd. for $C_{11}H_{19}NO_5$ (decarbethoxylated product): C, 53.88; H, 7.76.

(6) R. A. F. Bullerwell, A. Lawson and H. V. Morley, *J. Chem. Soc.*, 3283 (1954).

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An Explosion during the Preparation of Neopentyl Alcohol

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The preparation of neopentyl alcohol as recently described[1] has been repeated successfully many times in this Laboratory. Recently, however, a violent explosion occurred during a run. Investigation revealed that the explosion undoubtedly took place after the acetone peroxide which had been removed by suction filtration was allowed to be sucked dry on the funnel. The suction flask was unharmed but much damage resulted from the explosion. Accordingly, when this preparation is carried out as described,[1] care should be taken that the solid peroxide be kept moist and destroyed with care. Preferably, an alternate procedure which avoids the formation of acetone peroxide should be used.

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Preparation of Modified Squalenes

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There is considerable evidence that cholesterol plays an important, if ambiguous, role in the development of atherosclerosis.[1] Recent progress in the elucidation of the biogenesis of cholesterol has demonstrated that the hydrocarbon squalene is the most efficient precursor to cholesterol yet discovered.[2] It was our purpose to prepare modified squalenes in the hope that these compounds would act as metabolic antagonists to the endogenous syntheses of cholesterol. The reduction of the serum cholesterol concentration should have a beneficial effect on the severity of the atherosclerotic lesions.

It was shown recently that the hydrocarbon regenerated from squalene hexahydrochloride has a different structure from the natural squalene.[3] This can be differentiated from the natural material in biological systems.[4] We have investigated the preparation of squalene hexahydrobromides and particularly the effect of peroxide on the mode of addition of hydrogen bromide. It was hoped that materials more closely analogous to natural squalene could be regenerated from squalene hexahydrobromides of approxpiate structure. For example, a "squalene" differing from natural squalene in the position of one of the double bonds possibly could function as a metabolic antagonist to squalene utilization. We repeated the experiment of Heilbron and co-workers[5] and found, as these workers had foreshadowed, that a mixture of hexahydrobromides results when squalene is treated with hydrogen bromide in acetone solution. This mixture is rather readily separated by fractional crystallization into isomers melting at 112–114, 135–138, and 151–153°. Schmidt[6] reports the isolation of two hexahydrobromides, m.p. 116–118° and 136–138° from synthetic squalene. The infrared spectra of these isomers were similar, but distinctly different. When the hydrogen bromide reaction was run in the presence of 0.02 mole % of ascaridole a different result was obtained. A single hexahydrobromide

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(2) R. G. Langdon and K. Bloch, THIS JOURNAL, **74**, 1869 (1952).
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(6) J. Schmidt, *Ann.*, **547**, 115 (1941).