powdered gentian root of commerce,[5] which may depend upon the stage of development of the plant, has led to the suggestion that the best way to prepare gentiobiose is to synthesize it by treating 2,3,4,6-tetra-*O*-acetyl-α-glucosyl bromide with 1,2,3,4-tetra-*O*-acetyl-D-glucose. This provides the crystalline octaacetate[6,7] from which the free disaccharide may be obtained by deacetylation. A recent modification of this synthetic approach involving the interaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide with 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucose in the presence of silver perchlorate is worthy of note.[7] A biochemical synthesis effected by the action of almond emulsin (β-D-glucosidase) on D-glucose has also been recommended,[8] and recently controlled hydrolysis of yeast glucan[9] and of the β 1 → 6 linked D-glucan (pustulan) from *Umbilicaria pustulata*[10] has been shown to give gentiobiose.

During the summer of 1961, roots of the yellow gentian (*Gentiana lutea*) were collected in the area of Lausanne, Switzerland, and shown to contain gentiobiose in such amounts that acetylation of the 50% aqueous ethanol extract readily afforded crystalline gentiobiose octaacetate, the yield amounting to 23 g./kg. of dried roots.

Roots of a second species of gentian (*Gentiana andrewsii*) collected in September, 1963, in New Hampshire (U. S. A.) have also been found to be a good source of gentiobiose. In this case, the roots were extracted with water and the extract was treated with ethanol to precipitate a polysaccharide which was composed of arabinose, galactose, glucose, and traces of rhamnose. Acetylation of the mixture of sugars recovered from the aqueous ethanolic solution readily afforded gentiobiose β-octaacetate, the yield of the latter corresponding to 26 g./kg. of dried roots. Thus, treatment of the material with yeast invertase as formerly recommended[3-5] is unnecessary.

Experimental

All evaporations were carried out under reduced pressure at about 40°.

Isolation of Gentiobiose from Gentian Roots.—A. From *Gentiana lutea*. Roots were collected from flowering plants of the yellow gentian found in the vicinity of Lausanne, Switzerland, during the first week of July, 1961. The partially dried roots (150 g.) were cut into small pieces and extracted with 50% aqueous ethanol (900 ml.) at room temperature during 12 hr. The extract was decanted and the residue was ground in a mortar and a second extraction was carried out in the same manner. After three extractions had been made, the combined solutions were concentrated *in vacuo* at 40° to a volume of about 200 ml. This solution was treated with ethanol (400 ml.) and, after adding charcoal, the solution was filtered and concentrated to 100 ml. Paper chromatography showed that this solution contained glucose, sucrose, and gentiobiose, and smaller porportions of other slow moving components. The solution (100 ml.) was treated with water (100 ml.) and invertase (10 mg.) was added. Addition of invertase is believed to be unnecessary (see B below). After keeping overnight, the solution was concentrated to dryness and the residue was dissolved in pyridine (30 ml.) and treated with acetic anhydride (20 ml.) at room temperature for 12 hr. The reaction mixture was poured with stirring into water and after 2 hr. the product was extracted with chloroform (200 ml.) The chloroform solution was washed with water (three times), dried ($MgSO_4$), and concentrated to a sirup. This sirupy product was dissolved in warm methanol and after nucleating with β-gentiobiose octaacetate, the solution was allowed to crystallize. After keeping for 12 hr., filtration and washing gave a crude product (3.5 g.) which when recrystallized from methanol gave β-gentiobiose octaacetate, m.p. 192.5–194°, $[\alpha]^{25}D$ −5.6° (*c* 1.3, chloroform); lit.[5] (β-gentiobiose octaacetate) m.p. 193°, $[\alpha]D$ −5.3° (chloroform); yield 2.5 g. of octaacetate from 250 g. of gentian root of unspecified origin; lit.[5,7] (α-octaacetate) m.p. 188–189°, $[\alpha]D$ +52.4° (chloroform).

B. From *Gentiana andrewsii*.—Roots of this species of blue gentian were collected from flowering plants found in the mountains of New Hampshire, U. S. A., in September, 1963. The partially dried roots (77 g.) which had been kept at room temperature for about 7 days were heated with water (500 ml.) on a steam bath for 5 hr. The swollen roots were disintegrated in a Waring Blendor in the presence of added water (total volume 1000 ml.). The mixture was filtered through a linen cloth and the filtrate was concentrated to a volume of 500 ml. and treated with ethanol (1000 ml.). The polysaccharide which was precipitated at this stage was recovered (centrifuge) and purified by reprecipitation (twice) from aqueous solution with ethanol and then dried *in vacuo* after washing successively with ethanol, ether, and petroleum ether. This polysaccharide, which readily dissolved in water, showed $[\alpha]^{24}D$ +157° (*c* 1, water), and, upon hydrolysis by heating (sealed tube) with 0.5 *N* H_2SO_4 for 5 hr. in a boiling water bath, it gave rise to arabinose, galactose, glucose, and traces of rhamnose as revealed by paper chromatography.

Evaporation of the aqueous ethanolic solution from the first precipitation of the polysaccharide gave a sirupy product which was dissolved in methanol (250 ml.). After removing a small proportion of insoluble precipitate, the methanolic solution was concentrated to dryness and the yellowish brown residue was subjected to acetylation by heating for 2 hr. with a mixture of acetic anhydride (135 ml.) and anhydrous sodium acetate (11 g.). The reaction mixture was poured with stirring into water (1000 ml.) and, after the excess of acetic anhydride had decomposed, the acetylated product was extracted with chloroform (500 ml.). The chloroform solution was washed with an aqueous solution of sodium bicarbonate and with water. The dried ($MgSO_4$) chloroform extract was treated with charcoal, filtered, and concentrated to remove the solvent. The residue was dissolved in a small volume of methanol and the solution, after nucleation with β-gentiobiose octaacetate, was kept at room temperature for 2 days until crystallization was complete. The crystalline mass was diluted with methanol and the crystals were recovered by filtration. Recrystallization of the product from methanol gave β-gentiobiose octaacetate (2.0 g.), m.p. 195° and $[\alpha]^{25}D$ −6.4° (*c* 2, chloroform).

Acknowledgment.—The authors thank the Corn Industries Research Foundation for financial support.

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Synthesis of α-Keto Acids from Diethyl Alkylidenemalonates

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Received November 13, 1963

An earlier paper[1] described the preparation of α-keto amides by epoxidation of ethyl alkylidenecyanoacetates and subsequent decarboxylation of the epoxy acids thus obtained. This procedure has now been extended to the synthesis of α-keto acids by the use of diethyl alkylidenemalonates.

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TABLE I

EPOXIDATION OF DIETHYL ALKYLIDENEMALONATES

Diethyl alkylidenemalonate	Epoxy compound	B.p., °C. (mm.)	Yield, %	Formula	Anal., % Calcd. C	Calcd. H	Found C	Found H
Propylidene[a]	IIa	123–129 (8)	64	$C_{10}H_{16}O_5$	55.54	7.46	55.61	7.32
Butylidene[a]	IIb	130–133 (6)	75[b]	$C_{11}H_{18}O_5$	57.38	7.88	57.42	7.51
Isobutylidene[a]	IIc	120–122 (6)	72	$C_{11}H_{18}O_5$	57.38	7.88	57.73	7.89
Isopentylidene[a]	IId	129–133 (5)	58	$C_{12}H_{20}O_5$	59.00	8.25	59.34	8.15
n-Hexylidene[a]	IIe	150–155 (7)	70	$C_{13}H_{22}O_5$	60.44	8.59	60.77	8.40
n-Heptylidene[c]	IIf	153–157 (6)	72	$C_{14}H_{24}O_5$	61.74	8.88	62.00	8.63

[a] Ref. 2. [b] When trisodium phosphate and potassium carbonate as a catalyst were used, the yields were 37 and 3%, respectively. [c] B. Wojcik and H. Adkins, *J. Am. Chem. Soc.*, **56**, 2424 (1934).

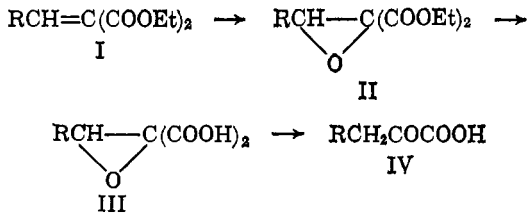
TABLE II

PREPARATION OF α-KETO ACIDS FROM EPOXY ESTERS (II)

α-Keto acid	B.p. (mm.) or m.p., °C.	Yield,[a] %	Formula	Anal., % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
IVa	58–61 (5)[b]	42 (A)	$C_5H_8O_3$	51.72	6.94		51.27	6.80	
Oxime	143 dec.[c]		$C_5H_9NO_3$			10.68			10.60
IVb	99–102 (20)[d]	46 (A) 35 (B)	$C_6H_{10}O_3$	55.37	7.75		54.98	7.56	
Oxime	132 dec.[e]		$C_6H_{11}NO_3$			9.65			9.43
IVc	97–98 (26)[f]	60 (A) 47 (B)	$C_6H_{10}O_3$	55.37	7.75		55.21	7.63	
Oxime	148 dec.[g]		$C_6H_{11}NO_3$			9.65			9.57
IVd	98–100 (11)[h]	58 (B)	$C_7H_{12}O_3$	58.31	8.39		57.89	8.01	
IVe	101–103 (4)[i]	65 (B)	$C_8H_{14}O_3$	60.74	8.92		60.33	8.76	
IVf	41–42[j]	60 (B)	$C_9H_{16}O_3$	62.76	9.36		62.57	9.22	
Oxime	97[k]		$C_9H_{17}NO_3$			7.48			7.34

[a] Capital letters refer to decarboxylation methods designated by these letters in the Experimental section. [b] F. Adickes and G. Andresen [*Ann.*, **555**, 41 (1944)] report b.p. 66° (6 mm.). [c] K. E. Hamlin and W. H. Hartung [*J. Biol. Chem.*, **145**, 351 (1942)] report m.p. 145° dec. [d] Lit.[b] b.p. 101–102° (20 mm.). [e] Lit.[b] m.p. 132–133° dec. [f] J. Schreiber [*Ann. Chem.* (Paris), [12]**2**, 98 (1947)] reports b.p. 84° (15 mm.). [g] T. Uyemura [*Bull. Agr. Chem. Soc. Japan*, **15**, 353 (1939)] reports m.p. 147° dec. [h] R. Locquin [*Bull. soc. chim. France*, [3]**31**, 1153 (1904)] reports b.p. 101–102° (12 mm.). [i] Lit.[b] b.p. 118–123° (13 mm.). [j] Lit.[b] m.p. 43–44°. [k] Lit.[b] m.p. 98–98.5°.

Diethyl alkylidenemalonates (I) have been prepared in good yield by using the conditions developed by Cope, *et al.*[2] Epoxidation of these unsaturated esters with hydrogen peroxide proceeded smoothly in the presence of sodium tungstate to give the epoxy esters (II). Alkaline hydrolysis of the epoxy esters afforded the corresponding acids (III) which were readily decarboxylated to α-keto acids (IV).



a, R = C_2H_5 c, R = *i*-C_3H_7 e, R = *n*-C_5H_{11}
b, R = *n*-C_3H_7 d, R = *i*-C_4H_9 f, R = *n*-C_6H_{13}

First, butyraldehyde was condensed with diethyl malonate, and the resulting diethyl butylidenemalonate was treated with 30% hydrogen peroxide at 70–80° giving ethyl 2,3-epoxy-2-ethoxycarbonylcaproate (IIb).

Payne[3] showed that ethanolysis of ethyl 2,3-epoxy-2-ethoxycarbonylbutyrate leads to ethyl 3-ethoxy-2-ethoxycarbonyl-2-hydroxybutyrate or its isomer, ethyl 2-ethoxy-2-ethoxycarbonyl-3-hydroxybutyrate. When the ethanolysis was carried out with IIb, the product was a mixture of ethyl 2-ethoxy-2-ethoxycarbonyl-3-hydroxycaproate and ethyl 3-ethoxy-2-ethoxycarbonyl-2-hydroxycaproate.

The epoxy ester (IIb), on treatment with alcoholic potassium hydroxide, gave a potassium salt of 2-carboxy-2,3-epoxycaproic acid. The epoxy acid produced by acidification of the salt was decarboxylated on heating in an oil bath, giving 2-oxocaproic acid, identical (physical constants, infrared spectrum, and oxime derivative) with the compound prepared previously from α-oxocaproamide.[1]

These techniques have also been successfully applied to other α,β-unsaturated diesters. These results are summarized in Tables I and II.

An attempt was then made to modify the above approach to diethyl 3-methylpropylidenemalonate. The ester was recovered unchanged, however, after prolonged treatment with 30% hydrogen peroxide. This result is in accord with the observation of Payne.[3] He has reported that, whereas diethyl ethylidenemalonate on treatment with 50% hydrogen peroxide gave the epoxy compound in high yield, diethyl isopropylidenemalonate under similar conditions failed to undergo epoxidation because of a steric inhibition of coplanarity.

A convenient and direct synthesis of α-keto esters from epoxy esters (II) was also investigated. Ethyl

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(3) G. B. Payne, *J. Org. Chem.*, **24**, 2048 (1959).

2,3-epoxy-2-ethoxycarbonylcaproate (IIb) and 0.5 mole of alkali in ethanol at room temperature afforded ethyl 2-carboxy-2,3-epoxycaproate. The monoester was converted, by heating, into the known ethyl 2-oxocaproate, on decarboxylation and rearrangement of oxygen. In a similar reaction sequence, isopropyl 4-methyl-2-oxovalerate was prepared in good yield from isopropyl 2,3-epoxy-2-ethoxycarbonyl-4-methylvalerate.

In an attempt to obtain α-keto esters from ethyl 2-carbamoyl-2,3-epoxycarboxylate,[1] selective hydrolysis of the carbamoyl group by Fischer's procedure[4] was examined. Reaction of ethyl 2-carbamoyl-2,3-epoxy-3-methylvalerate with nitrous acid in ether at 0° gave a 50% yield of ethyl 2-carboxy-2,3-epoxy-3-methylvalerate which could be converted into ethyl 3-methyl-2-oxovalerate on decarboxylation.

Experimental

General Procedure. Epoxidation.—Diethyl alkylidenemalonate (0.01 mole), 15 ml. of 30% hydrogen peroxide, 15 ml. of ethanol, and 0.7 g. of sodium tungstate dihydrate were placed in a round-bottom flask, fitted with a reflux condenser and thermometer. The mixture was heated to 70–80° on a water bath for about 1 hr. After an additional hour, ethanol was removed by distillation. The oily layer was separated from the aqueous layer, the aqueous layer was extracted with two portions of ether, and the combined extracts and oil were dried over calcium chloride. The ether was removed by distillation. Fractional distillation of the residue gave epoxy ester (II).

Hydrolysis.—The epoxy ester (II) was dissolved in an ethanolic solution of potassium hydroxide and allowed to saponify overnight at room temperature (or elevated temperature). The resulting precipitate was dissolved in water and acidified with dilute hydrochloric acid. The aqueous solution was extracted with ether. The ether was distilled and all low-boiling material was removed from the residue on a water bath under reduced pressure. The epoxy acid which remained in the flask was used without further purification for the preparation of α-keto acid. The yields of crude epoxy acids were 80–90%.

Decarboxylation. A.—In a Claisen flask was placed the epoxy acid. When it was heated in an oil bath at 180–200° under reduced pressure, evolution of carbon dioxide was observed. The product was removed by distillation. The distillate was dissolved in an aqueous sodium carbonate solution and then shaken with ether. The alkali solution was acidified with dilute hydrochloric acid and again extracted with ether. The ether was removed by distillation. Fractional distillation of the residue gave α-keto acid.

B.—Epoxy acid (III) was heated with 50% sulfuric acid for 2–3 hr. The solution was cooled and extracted with ether. The ethereal solution was shaken with an aqueous sodium carbonate solution. The aqueous layer was acidified and then extracted with ether. Fractional distillation of the extract gave α-keto acid.

Ethyl 2-Oxocaproate from Ethyl 2,3-Epoxy-2-ethoxycarbonylcaproate (IIb).—In a three-necked flask equipped with a sealed stirrer, dropping funnel, and a reflux condenser, 24.5 g. of ethyl 2,3-epoxy-2-ethoxycarbonylcaproate (IIb) and 100 ml. of ethanol were charged, and a solution of 5.6 g. of potassium hydroxide in 60 ml. of ethanol was added at room temperature with stirring. After the mixture had stood overnight, it was heated to boiling on a water bath and filtered. A sirup was obtained by concentrating the mother liquor. The sirup was dissolved in water and shaken with ether. The aqueous layer was cooled to 5° and a slight excess of hydrochloric acid was added while the temperature was maintained below 10°. The aqueous solution was then extracted with ether. The ethereal solution was concentrated to give the monoethyl ester. The yield was 15 g. The product was used without further purification for the following reaction.

Decarboxylation of the monoester was carried out as in the above experiment A. The fraction distilling at 103–110° (35 mm.) was collected (10 g.), lit.[5] b.p. 83.5–84° (10 mm.).

Anal. Calcd. for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.41; H, 8.80.

2,4-Dinitrophenylhydrazone had m.p. 120°, lit.[5] m.p. 120.5–121°.

Anal. Calcd. for $C_{14}H_{18}N_4O_6$: N, 16.56. Found: N, 16.24.

Ethyl isopropyl isobutylidenemalonate was prepared by Cope's method[2] from ethyl isopropyl malonate and isobutyraldehyde. The yield was 87%, b.p. 106–107° (4 mm.).

Anal. Calcd. for $C_{12}H_{20}O_4$: C, 63.13; H, 8.83. Found: C, 62.89; H, 8.63.

Isopropyl 2,3-Epoxy-2-ethoxycarbonyl-4-methylvalerate.—Epoxidation of ethyl isopropyl isobutylidenemalonate was carried out, by using trisodium phosphate, as described above. The fraction distilling at 115–116° (4 mm.) was collected. The yield was 70%.

Anal. Calcd. for $C_{12}H_{20}O_5$: C, 59.00; H, 8.25. Found: C, 58.26; H, 8.23.

Isopropyl 4-Methyl-2-oxovalerate.—Hydrolysis of isopropyl 2,3-epoxy-2-ethoxycarbonyl-4-methylvalerate was carried out as above. The resulting crude isopropyl 2-carboxy-2,3-epoxy-4-methylvalerate was heated at 180–200° in an oil bath, and then the fraction distilling at 88–89° (23 mm.) was collected. The yield was 51%.

Anal. Calcd. for $C_9H_{16}O_3$: C, 62.76; H, 9.36. Found: C, 62.09; H, 9.11.

The product gave a green color with ferric chloride in ethanol.

Ethyl 3-Methyl-2-oxovalerate from Ethyl 2-Carbamoyl-2,3-epoxy-3-methylvalerate.[1]—Ten grams of ethyl 2-carbamoyl-2,3-epoxy-3-methylvalerate was dissolved in 75 ml. of ether containing 5 ml. of water and then the solution was cooled in an ice bath. A steady stream of nitrous acid was introduced into the cold solution while cooling was continued for a period of 14 hr. The ethereal solution was then washed with water and the ether was removed by distillation. The residue was dissolved in an aqueous sodium hydrogen carbonate solution, and the alkaline solution was extracted with ether to remove any material not soluble in alkali. The aqueous solution was then acidified with dilute hydrochloric acid and again extracted with ether. The ether was removed to give the crude product. The above purification procedure was repeated. Ethyl 2-carboxy-2,3-epoxy-3-methylvalerate was obtained in 50% yield as an oil.

Anal. Calcd. for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.01; H, 6.77.

The compound was decarboxylated by heating in an oil bath (bath temperature, 180–190°) giving ethyl 3-methyl-2-oxovalerate, 2 g., b.p. 75–80° (15 mm.), lit.[6] b.p. 78–79° (15 mm.).

Anal. Calcd. for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.31; H, 8.44.

The ester gave a dark green color with ferric chloride in ethanol.

Ethyl 4-Methyl-2-oxovalerate from Ethyl 2-Carbamoyl-2,3-epoxy-4-methylvalerate.[1]—The procedure was carried out as above. Ethyl 4-methyl-2-oxovalerate, b.p. 100–104° (35 mm.), was obtained in 42% yield, lit.[5] b.p. 76–77° (10 mm.).

2,4-Dinitrophenylhydrazone had m.p. 79°, lit.[5] m.p. 79.5–80°.

Anal. Calcd. for $C_{14}H_{18}N_4O_6$: N, 16.56. Found: N, 16.31.

Ethanolysis of Ethyl 2,3-Epoxy-2-ethoxycarbonylcaproate (IIb).—A solution of 4.4 g. of IIb in 25 ml. of ethanol containing 1 ml. of concentrated sulfuric acid was refluxed for 8 hr. The solution was then neutralized with calcium carbonate. After filtration and concentration, the resulting residue was taken up in warm ether and filtered. Distillation of the filtrate gave 4.0 g. of material, b.p. 136–138° (6 mm.), having an analysis in agreement with ethyl 2-ethoxy-2-ethoxycarbonyl-3-hydroxycaproate and its isomer. Hell-Uroch's, Diniges', Chancel's, and Nessler's reactions[7] were all positive.

Anal. Calcd. for $C_{12}H_{24}O_4$: C, 62.04; H, 10.41. Found: C, 61.46; H, 10.31.

Acknowledgment.—The authors wish to express their sincere thanks to Dr. Taro Hayashi and Dr. Tatsuo Takeshima for their kind advice. Our study was aided by a grant from the Scientific Research Fund of the Ministry of Education.

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