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BIOCHEMISTRY OF THE USTILAGINALES

VIII. THE STRUCTURES AND CONFIGURATIONS OF THE USTILIC ACIDS[1]

By R. U. Lemieux

ABSTRACT

Methanolysis of ustilagic acid and hydrolysis of the methyl esters formed yielded a crystalline acidic fraction which was essentially a mixture of two substances termed the ustilic acids A and B. The acids were separated as their isopropylidene derivatives. The ustilic acids cocrystallize to mixtures with melting points intermediate between those of the pure compounds. Conversion of ustilic acid A, m.p. 112–113° C., $[\alpha]_D$ $-8°$ in methanol, which made up about 70% of the mixture, by hydrogenolysis to palmitic acid, by oxidation with chromic oxide to pentadecanedioic acid, and by lead tetraacetate oxidation followed by hydrogenation to 15-hydroxypentadecanoic acid showed the substance to be an optically active form of 15,16-dihydroxyhexadecanoic acid. Conversion of ustilic acid B, m.p. 140–141° C., $[\alpha]_D$ $-10°$ in methanol, by sodium bismuthate oxidation followed by hydrogenation to 1,14-dihydroxytetradecane, by chromic acid oxidation of its methyl ester followed by hydrolysis of the product, and peroxide oxidation of the α-keto acid thus formed to tetradecanedioic acid, and by hydrogenolysis of the C2-carbon atom through a series of reactions to ustilic acid A, showed the substance to be an optically active form of 2,15,16-trihydroxyhexadecanoic acid. Optically active forms of 2,15-dihydroxypentadecanoic and 2-hydroxypentadecanoic acids were prepared from ustilic acid B. Application of certain empirical rules of rotation to derivatives of these 2-hydroxyacids showed them to possess the D-configuration. Reduction of ustilic acid B with lithium aluminum hydride gave *meso*-1,2,15,16-tetrahydroxyhexadecane. Thus, ustilic acid B was the 2D,15D,16-trihydroxyhexadecanoic acid and the ustilic acid A was the 15D,16-dihydroxyhexadecanoic acid. Several derivatives of the above described acids were prepared.

INTRODUCTION

The new antibiotic (8) termed ustilagic acid was shown (15) to be a mixture of closely related, partially acylated D-glucolipides. Alkaline hydrolysis of ustilagic acid yielded an amorphous substance designated glucoustilic acid and removal of the glucose residues in the latter material yielded a crystalline substance termed ustilic acid (15).

Repeated crystallizations of crude methyl ustilate from methanol yielded a substance with the composition expected for the methyl ester of a dihydroxyhexadecanoic acid and hydrolysis of this ester gave a ustilic acid, m.p. 114–115° C. (15). It was later found, however, that if the crude methyl ustilate, obtained on the methanolysis of glucoustilic acid, was not subjected to purification prior to hydrolysis a ustilic acid preparation was obtained which melted at a higher temperature, in the range 120–126° C., than the above dihydroxyhexadecanoic acid. Furthermore, repeated recrystallizations of the crude ustilic acid did not yield the latter substance but instead gave higher melting materials of indeterminate composition. The infrared spectrum of recrystallized ustilic acid mixture (see Fig. 1) was much different to that of the acid with melting point 114–115° C. and it was therefore apparent that crude ustilic acid was a mixture.

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[1] *Manuscript received in original form June 30, 1952, and, as revised, January 9, 1953.*
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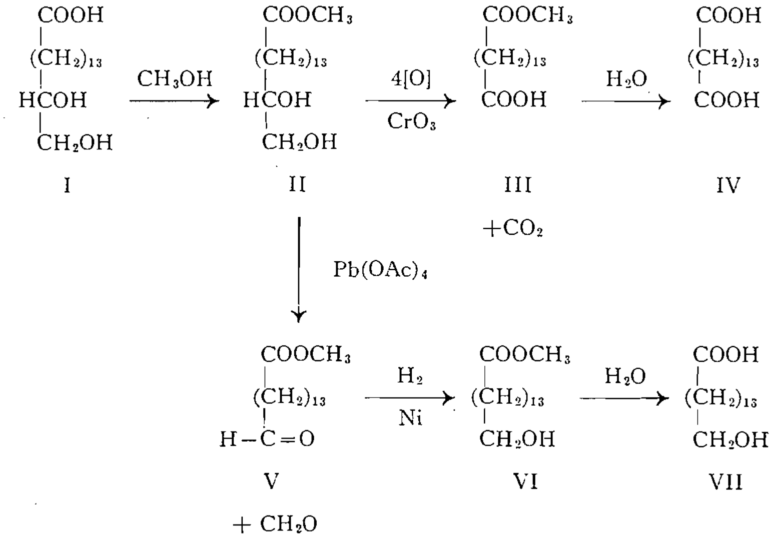
Methanolysis of 17.2 me. of ustilagic acid, neutral equivalent 801 and saponification equivalent 396, which contained 1.01 acetyl, 0.67 3L-hydroxyhexanoyl, 0.27 3L-hydroxyoctanoyl, 0.05 hexanoyl groups, and 1.96 glucose residues per neutral equivalent, and hydrolysis of the methyl ustilate fraction yielded 17.4 me. of crude ustilic acid, m.p. 122–126° C., with neutral equivalent 296. Periodate oxidation of the crude ustilic acid liberated one mole of formaldehyde per neutral equivalent (see Table II) and it was thus apparent that the components of the ustilic acid mixture contained, on the average, one terminal α-glycol group. Only a trace of steam-volatile acidity was formed in terminal methyl group assays and the ustilic acids therefore appeared free of CH_3–C groups.

Treatment of the crude ustilic acid mixture with dry acetone containing sulphuric acid yielded a mixture of isopropylidene derivatives. On converting the acid isopropylidene derivatives to methyl ester using diazomethane, it was noticed that considerably less than the calculated amount of diazomethane was required. The esters thus formed were found to possess an isopropylidene group content, 15.3%, which was higher than that which would be expected if the ustilic acids had formed monoisopropylidene derivatives. Furthermore, treatment of the mixture with alkali liberated acetone and it was therefore apparent that the material contained carboxyl groups which were esterified through isopropylidene group linkages. Fractional distillation at about 0.05 mm. pressure separated the mixture of esters, 4.20 gm., into two main fractions; 2.52 gm., b.p. 133–136° C. and 1.16 gm., b.p. 154–156° C. It was thus established that the lower boiling material was a derivative of the major component of the ustilic acid mixture.

Purification of the above described lower boiling fraction by recrystallization from methanol gave a material, m.p. 46–47° C., $[\alpha]_D$ +12° in chloroform, with the composition expected for a methyl isopropylidenedihydroxyhexadecanoate. Methanolysis gave a compound, m.p. 85.5–86° C., $[\alpha]_D$ −7° in methanol, with the composition expected for a methyl dihydroxyhexadecanoate, and hydrolysis of this ester yielded a dihydroxyhexadecanoic acid, m.p. 112–113° C., $[\alpha]_D$ −8° in methanol. This acid is a purer sample of the previously described (15) ustilic acid, m.p. 114–115° C. and is designated ustilic acid A (17). The substance is further characterized by its infrared spectrum (see Fig. 1) and its hydrazide derivative, m.p. 140.5–141.5° C.

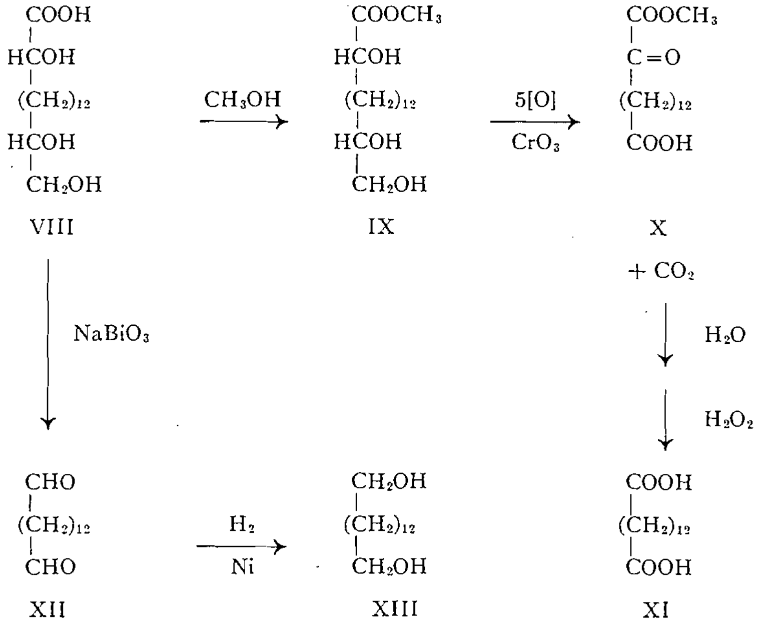
Purification of the above described higher boiling fraction by recrystallization from methanol gave a material, m.p. 42.5–43° C., $[\alpha]_D$ +18° in chloroform, with the composition expected for a diisopropylidenetrihydroxyhexadecanoate. Methanolysis of this compound yielded a substance, m.p. 74–75° C., $[\alpha]_D$ −8.5° in methanol, with the composition expected for a methyl trihydroxyhexadecanoate, and hydrolysis of this ester yielded a new trihydroxyhexadecanoic acid, m.p. 140–141° C., $[\alpha]_D$ −10° in methanol, termed ustilic acid B. The substance is further characterized by its infrared spectrum (see Fig. 1) and its hydrazide derivative, m.p. 164–165° C.

Treatment of ustilic acid A with hydrogen iodide and hydrogenation of the product in alkaline solution in the presence of Raney nickel gave an acid with a melting point, 59–61° C., which was not depressed by admixture of palmitic acid, m.p. 61.5–62° C. Periodate oxidation of ustilic acid A liberated one mole of formaldehyde, characterized through its methone derivative and determined colorimetrically using chromotropic acid (14). It was thus apparent that ustilic acid A was an optical isomer of 15,16-dihydroxyhexadecanoic acid (I). This conclusion was confirmed by the following observations. Oxidant equivalent to four atoms of oxygen was rapidly consumed on oxidation of methyl ustilate A (II) with chromium trioxide in acetic acid solution. Hydrolysis of the product (III) gave the known pentadecanedioic acid (IV) (4). Oxidation of methyl ustilate A (II) with lead tetraacetate yielded an aldehyde (V) which on hydrogenation gave the methyl ester (VI) (5) of the known 15-hydroxypentadecanoic acid (VII) (3). 15-Hydroxypentadecanohydrazide melted at 142.5–143.5° C.



Oxidant equivalent to five atoms of oxygen was rapidly consumed on oxidation of methyl ustilate B (IX) with chromium trioxide in glacial acetic acid. Hydrolysis of the product (X), m.p. 87–87.5° C., and oxidation with hydrogen peroxide yielded a substance with the composition and melting point of the known tetradecanedioic acid (XI) (4). It was thus apparent that ustilic acid B was an optically active form of 2,15,16-trihydroxyhexadecanoic acid (VIII). This conclusion was confirmed by oxidation of ustilic acid B with sodium bismuthate in glacial acetic acid (28) to the dialdehyde XII which was converted by hydrogenation to a compound with the melting point and composition expected for the known 1,14-dihydroxytetradecane (XIII) (4).

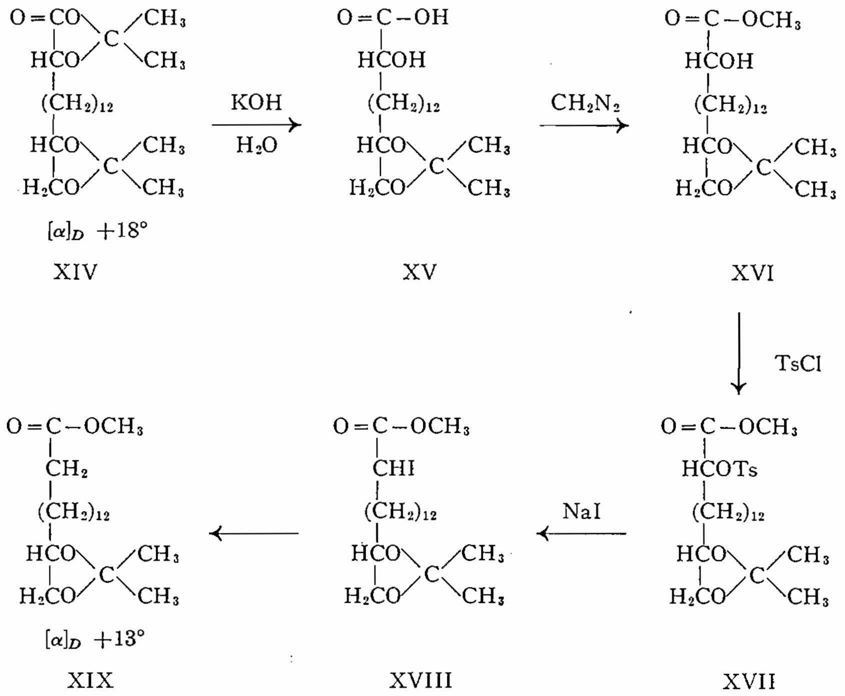
A convenient method for the separation of the ustilic acids was based on the fact that, while ustilic acid A yielded an acidic monoisopropylidene derivative, ustilic acid B yielded a neutral diisopropylidene derivative. The extraction procedure was based on the method used for the determination of the quantity of neutral oil in acidic crude oils described by Jamieson (12).



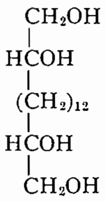
A mixed melting point curve was determined for the ustilic acids A and B by mixing weighed amounts of the materials and, after grinding the mixture in a mortar, determining its melting point. There was a continual rise in melting point with increasing amount of ustilic acid B such that the melting point curve appeared to be roughly a straight line joining the melting points of the two substances.

Alkaline hydrolysis of diisopropylideneustilate B (XIV) and methylation of the product (XV) with diazomethane yielded methyl 15,16-isopropylideneustilate B (XVI), m.p. 42.5–43° C., $[\alpha]_D$ +6° in chloroform. Tosylation of XVI and treatment of the product (XVII) with sodium iodide in acetone and hydrogenolysis of the iodocompound formed (XVIII) gave a material from which methyl isopropylideneustilate A (XIX) was prepared. It was thus established that the C15-carbon atoms of the ustilic acids A and B possess identical relative configurations. It is noteworthy that this conclusion is in accord with that expected on the basis of a comparison of the rotations, measured in chloroform, of methyl isopropylideneustilate A (XIX), $[\alpha]_D$ +12°, diisopropylideneustilate B (XIV), $[\alpha]_D$ +18°, and 1,2-isopropylidene-2,15-dihydroxypentadecanoate (XXVIII, see below), $[\alpha]_D$ +5°.

Reduction of diisopropylideneustilate B (XIV) with lithium aluminum hydride and hydrolysis of the product yielded *meso*-1,2,15,16-tetrahydroxyhexadecane (XX), m.p. 133–134° C. The structure of this substance was confirmed by the observation that it rapidly consumed two moles of lead tetraacetate and its optical inactivity was substantiated by the preparation of optically inactive diisopropylidene and tetraacetyl derivatives, XXI and XXII, respectively. Under the conditions of measurement used, XX appeared to be very slightly levorotatory. This observation indicates that the substance

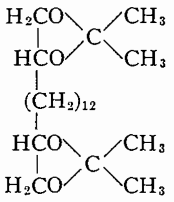


contained an optically active impurity. It is well known that α-hydroxyacids can undergo epimerization relatively easily and it seems likely that the saponification of methyl ustilate B in the preparation of ustilic acid B would be accompanied by the formation of a diastereoisomer which would be extremely difficult to completely separate from ustilic acid B. Therefore, the C2- and C15-hydroxyl groups of ustilic acid B were configurationally *cis* and a Fischer projection formula for ustilic acid B would possess these two hydroxyl groups on the same side of the carbon skeleton.

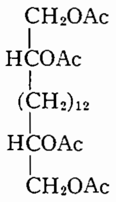


$$\begin{array}{c} CH_2OH \\ | \\ HCOH \\ | \\ (CH_2)_{12} \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

XX



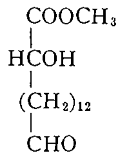
XXI



$$\begin{array}{c} CH_2OAc \\ | \\ HCOAc \\ | \\ (CH_2)_{12} \\ | \\ HCOAc \\ | \\ CH_2OAc \end{array}$$

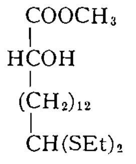
XXII

Oxidation of methyl ustilate B (IX) with lead tetraacetate yielded methyl 2-hydroxyaldehydopentadecanoate (XXIII) which was converted to the diethyldithioacetal (XXIV). Reductive desulphurization of the latter compound and hydrolysis of the product gave levorotatory 2-hydroxypentadecanoic acid (XXV), m.p. 89.5–90° C., $[\alpha]_D$ −0.8° in ethanol, which possessed a dextrorotatory sodium salt (see Table I). Hydrogenation of the aldehyde (XXIII) yielded the methyl ester (XXVI), m.p. 74.3–75.3° C., of weakly levorotatory 2,15-dihydroxypentadecanoic acid (XXVII), m.p. 102–103° C. Treatment of the latter compound with acetone and sulphuric acid afforded the dextrorotatory isopropylidene derivative XXVIII, m.p. 49–50° C., $[\alpha]_D$ $+5°$ in chloroform.



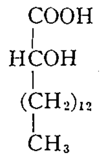
$$\begin{array}{c} COOCH_3 \\ | \\ HCOH \\ | \\ (CH_2)_{12} \\ | \\ CHO \end{array}$$

XXIII



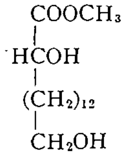
$$\begin{array}{c} COOCH_3 \\ | \\ HCOH \\ | \\ (CH_2)_{12} \\ | \\ CH(SEt)_2 \end{array}$$

XXIV



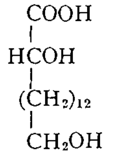
$$\begin{array}{c} COOH \\ | \\ HCOH \\ | \\ (CH_2)_{12} \\ | \\ CH_3 \end{array}$$

XXV



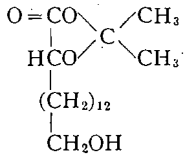
$$\begin{array}{c} COOCH_3 \\ | \\ HCOH \\ | \\ (CH_2)_{12} \\ | \\ CH_2OH \end{array}$$

XXVI



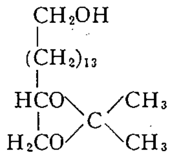
$$\begin{array}{c} COOH \\ | \\ HCOH \\ | \\ (CH_2)_{12} \\ | \\ CH_2OH \end{array}$$

XXVII

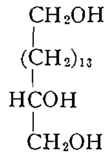


XXVIII

Reduction of methyl isopropylideneustilate A (XIX) with lithium aluminum hydride yielded the dextrorotatory isopropylidene derivative (XXIX), m.p. 60–61° C., $[\alpha]_D$ $+12°$ in chloroform, of levorotatory 1,15,16-trihydroxyhexadecane (XXX), m.p. 108.5–109° C., $[\alpha]_D$ $-7°$ in methanol. The triacetyl derivative (XXXI) of the triol melted at 24–25° C. with $[\alpha]_D$ $-0.8°$ in chloroform.

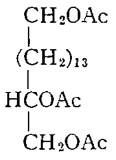


XXIX



$$\begin{array}{c} CH_2OH \\ | \\ (CH_2)_{13} \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

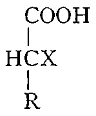
XXX



$$\begin{array}{c} CH_2OAc \\ | \\ (CH_2)_{13} \\ | \\ HCOAc \\ | \\ CH_2OAc \end{array}$$

XXXI

Table I lists the rotations of certain derivatives of the ustilic acids and of related compounds of known configuration. A comparison of these values allows a decision as to the configurations of the ustilic acids. The configurations are assigned on the basis of the conventions for nomenclature which were discussed in an earlier paper in this series (18). Levene and Kuna (21) have pointed out that the molar rotation of a given acid of the general type XXXII, in which X is a functional group and R is an alkyl group, will vary not only with variations in X but also with variations in R. Levene and Marker (22) have shown that the molar rotations of successive members of a configuration-



$$\begin{array}{c} COOH \\ | \\ HCX \\ | \\ R \end{array}$$

XXXII

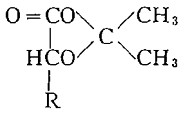
TABLE I

OPTICAL ROTATIONS OF USTILIC ACID DERIVATIVES AND RELATED COMPOUNDS

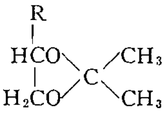
Compound	Mol. wt.	$[M]_D$	° C.	c	Solvent	Reference
A. Free acids						
1. 2L-Hydroxypropanoic	90	+340	25	..	H_2O	21
2. 2L-Hydroxybutanoic	104	+370	25	..	"	21
3. 2L-Hydroxyhexanoic	118	+500	25	..	"	21
4. 2D-Hydroxypentadecanoic	258	−206	..	5	C_2H_5OH	..
5. 2D-Hydroxyhexadecanoic	272	−272	..	5.2	"	13
B. Sodium salts						
1. 2L-Hydroxypropanoate	112	−1400	..	0.84	H_2O	27
2. 2L-Hydroxypentanoate	140	−2040	..	1	"	..
		−2310	..	1	25% C_2H_5OH	..
		−2900	..	1	50% "	..
		−3380	..	1	75% "	..
		−3700	..	1	C_2H_5OH	..
3. 2L-Hydroxyhexanoate	154	−2300	25	..	H_2O	21
4. 2D-Hydroxypentadecanoate	280	+4200	..	1	50% C_2H_5OH	..
C. Hydrazide						
2D,15-Dihydroxypentadecanohydrazide	288	+6930	..	0.2	50% C_2H_5OH	..
D. Isopropylidene lactones						
1. 1,2D,3L,4-Diisopropylidenetetrarate	230	+14,500	17	2.1	$(CHCl_2)_2$	7
2. 1,2-Isopropylidene-2D,15-dihydroxypentadecanoate	314	+1570	..	0.8	$CHCl_3$	..
E. Isopropylidene ketals						
1. 2,3-Isopropylidene-1,2D,3-trihydroxypropane	132	+1430	20	15.2	C_6H_6	1
2. 1,2-Isopropylidene-1,2L,16-trihydroxyhexadecane	314	+2350	..	1	C_6H_6	..

ally related homologous series progressively move in the same direction. Thus, as shown by the first three acids listed in Table I, the molar rotations of the normal α-hydroxyacids increase with increasing molecular weight. It is not possible to state with certainty that the same relationship will hold for the rotations of these acids measured in ethanol. Nevertheless, it seems highly probable that the levorotatory 2-hydroxypentadecanoic acid prepared from ustilic acid B and the levorotatory 2-hydroxyhexadecanoic acid, rotations measured in ethanol, isolated from the wool wax of Merino sheep by Kuwata (13), possessed configurations opposite to the homologous dextrorotatory 2L-hydroxyacids, rotations measured in water. Levene (19) has pointed out that the sodium salts of aldonic acids are more dextrorotatory than the free acids when the 2-carbon atom has the D-configuration and this relationship is well established for the simple 2-hydroxyacids (20,24). As shown in Table I, the molar rotations measured in water of the sodium salts of normal 2-hydroxyacids increase with increasing molecular weight. Also, the molar rotations of sodium 2L-hydroxypentanoate in 0, 25, 50, 75, and 100% aqueous ethanol increased with increase in ethanol concentration. Thus, a comparison of the molar rotation, +4150, of the sodium 2-hydroxypentadecanoate and that, −2900, of sodium 2L-hydroxypentanoate (both measured in 50% ethanol), as well as the fact that the former substance is more dextrorotatory in 50%

ethanol than its free acid in ethanol, indicates that the C2-carbon atoms of the 2-hydroxypentadecanoic acid and its parent substance, ustilic acid B, possessed the D-configuration. Hudson (10) and Levene and Meyer (23) have shown that the phenylhydrazides of aldonic acids with D-C2-carbon atoms are dextrorotatory and Van Marle (29) has shown that this relationship applies to unsubstituted hydrazides when measured in water. Therefore, the dextrorotation, $[M]_{\mathrm{D}}$ +6930°, found for the 2,15-dihydroxypentadecanohydrazide derived from ustilic acid B confirms the above allocation of configuration. The established success of Hudson's Rules of Isorotation (11, p. 17; 16) in the correlation of rotation with configuration for lactol carbon atoms situated in ring structures leaves no doubt that configurationally related homologous compounds such as XXXIII and XXXIV would possess the same sign of rotation. Therefore, the fact, as seen in Table I, that

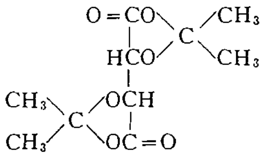


XXXIII



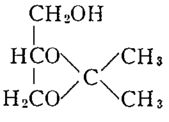
XXXIV

1,2D,3L,4-diisopropylidenetetrarate (XXXV) (7) is dextrorotatory supports the contention that the dextrorotatory 1,2-isopropylidene-2,15-dihydroxypentadecanoate (XXVIII) possessed the D-configuration. These arguments leave no doubt that ustilic acid B was the 2D,15D,16-trihydroxyhexadecanoic acid shown in VIII and that the configurationally related ustilic acid A was the 15D,16-dihydroxyhexadecanoic acid (I). Finally, the fact, as seen in Table I, that 2,3-isopropylidene-1-2D,3-trihydroxypropane (XXXVI) (1) is dextrorotatory indicates that the dextrorotatory 15,16-isopropylidene-1,15,16-trihydroxyhexadecane (XXIX) prepared from ustilic acid A possessed the same relative configuration, a conclusion which is in agreement with the above allocated configurational relationships. It is to be noted, however, that the presently widely accepted rules of nomenclature (6,18) infer that the substance XXX be oriented as shown in XXXVII for purposes of nomenclature. Thus, the asymmetric centers in the substances XXIX, XXX, and XXI, which were derived from 15D,16-dihydroxyhexadecanoic acid, possess the L-configuration. It is noteworthy that the system for nomenclature recently proposed by Cahn and Ingold (2) is free of such aberrations.

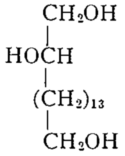


XXXV

The above described separation of the ustilic acids, by distillation of the esterified isopropylidene derivatives, indicated that the mixture contained



XXXVI



$$\begin{array}{c} CH_2OH \\ | \\ HOCH \\ | \\ (CH_2)_{13} \\ | \\ CH_2OH \end{array}$$

XXXVII

approximately twice as much ustilic acid A as ustilic acid B. Treatment of crude ustilic acid, neutral equivalent 296, obtained in quantitative yield from ustilagic acid, with acetone gave a purified ustilic acid mixture with neutral equivalent 293 in 96% yield. The calculated neutral equivalent for a mixture consisting of 70% ustilic acid A and 30% ustilic acid B is 293.2. This indication as to the composition of the ustilic acid mixture was confirmed by the yields of formaldehyde obtained on periodate oxidation of the crude and purified ustilic acid mixtures listed in Table II and by quantitative studies of the rates of oxidation by chromic oxide in acetic acid of methyl ustilate A, methyl ustilate B, and the mixture of methyl ustilates obtained on treatment of the purified ustilic acid mixture with diazomethane. The results of the latter studies are presented in Fig. 2. It is seen that the initial rapid uptake of oxidant by the methyl ustilates A and B, determined by extrapolation to zero time of the portions of the rate curves corresponding to the slow oxidation by the excess oxidant of the initial products, corresponded to 75.5 gm. (0.25 mole) and 63.6 gm. (0.20 mole) per atom of oxygen consumed, respectively. A similar extrapolation of the rate curve obtained on the oxidation of the methyl ustilate mixture gave a reduction equivalent per atom of oxygen of about 71 gm., the value to be expected if the methyl ustilate mixture were composed of 70% methyl ustilate A and 30% methyl ustilate B.

TABLE II

FORMATION OF FORMALDEHYDE ON PERIODATE OXIDATION OF USTILIC ACIDS

Time, hr.	Moles of formaldehyde formed per neutral equivalent of ustilic acid			
	Ustilic acid		Ustilic acid mixture	
	A	B	Crude	Purified
1	1.01	1.04	0.98	1.01
2	1.00	1.04	0.99	1.00
4	1.00	1.01	0.99	1.01

In a previous publication in this series (17) evidence was provided which indicated that in all probability the ustilagic acid mixture consists almost entirely of monoacetyl-mono-3L-hydroxyhexanoyl (65–70%) and monoacetyl-mono-3L-hydroxyoctanoyl (25–30%) substituted glucoustilic acids. Since the ustilic acid mixture was composed of about 70% ustilic acid A and 30% ustilic acid B, it seems highly probable that the mixture of ustilagic acids is composed essentially of two substances, ustilagic acid A (a monoacetyl-mono-

3L-hydroxyhexanoyl substituted di-β-D-glucosyl-15D,16-dihydroxyhexadecanoic acid) and ustilagic acid B (a monoacetyl-mono-3L-hydroxyoctanoyl substituted di-β-D-glucosyl-2D,15D,16-trihydroxyhexadecanoic acid). It is noteworthy that on the basis of these structures, ustilagic acid A possesses the molecular formula $(C_{2\cdot12}H_{3\cdot65}O)_{17}$ while the molecular formula for ustilagic acid B is $(C_{2\cdot11}H_{3\cdot67}O)_{18}$. This similarity in composition accounts, to a certain degree, for the difficulties encountered in the separation of these substances. The origin of the *n*-caproic acid found in the hydrolyzates of all ustilagic acid preparations (15, 17) remains obscure. Presumably this acid was derived from a third component which made up about 5% of the ustilagic acid mixture.

EXPERIMENTAL

Instrumentation

The infrared spectra and specific rotations were determined as previously described (15). The melting points are corrected.

Crude Ustilic Acid Mixture

Analysis (17) of the ustilagic acid sample used in this work showed it to contain 1.01 acetyl, 0.67 L-3-hydroxyhexanoyl, 0.27 L-3-hydroxyoctanoyl, and 0.05 hexanoyl groups and 1.96 glucose residues per neutral equivalent, 801. The saponification equivalent was 396.

A solution of the ustilagic acid, 13.8 gm. (17.2 me.), in 280 ml. of 4.3% methanolic hydrogen chloride was refluxed for 19 hr. The solution was poured into 600 ml. of water and the aqueous mixture was extracted with chloroform. The chloroform extract was dried and evaporated to a crystalline solid which was dissolved in 100 ml. of ethanol and 40 ml. of 2*N* potassium hydroxide. After the solution was refluxed for two hours, it was poured into 500 ml. of 0.2 *N* hydrochloric acid. The precipitate which formed was gathered by filtration and washed free of chloride with water. After it was dried *in vacuo* at 60° C., the tan-colored material, 5.16 gm. (17.4 me.), neutral equiv. 296, melted at 122–126° C. On the basis that one equivalent of ustilic acids should be obtained per equivalent of ustilagic acid, the yield of crude ustilic acid mixture was 101%.

Purification of the Ustilic Acid Mixture

The above described crude ustilic acid mixture, 590 mgm., was ground to a fine powder and refluxed with 5 ml. of dry acetone. After the mixture was cooled to −10° C., the ustilic acid precipitate was collected by filtration and washed with 5 ml. of cold dry acetone. Evaporation of the acetone filtrates yielded 24 mgm. of a darkly colored residue. Thus, the acetone extraction removed 4% of the ustilic acid mixture. After it was dried *in vacuo* at 60° C., the white ustilic acid mixture, neutral equivalent 293, melted at 123–128° C., with sintering at 120–123° C.

Methyl Ustilate Mixture

The above described purified ustilic acid mixture, 300 mgm., was dissolved in 20 ml. of methanol and the solution was treated with an excess of a freshly distilled ethereal solution of diazomethane. The excess diazomethane was removed by distillation and the solution was evaporated *in vacuo* to a white crystalline solid, m.p. 76–80° C., with sintering at 72–76° C.

Separation of Ustilic Acid Derivatives by Distillation

The above described crude ustilic acid mixture, 4.00 gm., was ground to a fine powder and shaken at room temperature for five hours with 100 ml. of dry acetone and eight drops of concentrated sulphuric acid (26). The ustilic acids, which are only sparingly soluble in acetone, soon dissolved. The yellow-colored solution was poured into a separatory funnel which contained 500 ml. of water and 100 ml. of chloroform. After the mixture was shaken for about 20 seconds, the chloroform layer was separated and washed with 200 ml. of water. The chloroform solution was dried by percolation through a column of dry Celite and evaporated *in vacuo* to a yellow oil which was treated with an excess of an ethereal solution of diazomethane. The ether was removed by distillation and the residue was dried at 0.1 mm. and 100° C. The yellow-colored product which crystallized at room temperature weighed 4.24 gm. and contained 15.3% isopropylidene groups. Fractional distillation of the material, 4.20 gm., at about 0.05 mm. pressure, yielded three fractions. The first fraction, b.p. 133–136° C., weighed 2.52 gm. and contained 12.8% isopropylidene groups. The second fraction was an intermediate fraction, b.p. 136–154° C. which contained 15.4% isopropylidene groups and weighed 0.27 gm. The third fraction distilled at 154–156° C., weighed 1.16 gm., and contained 20.5% isopropylidene groups.

Methyl Isopropylideneustilate A

The first fraction from the above distillation was recrystallized twice from methanol to yield a material, m.p. 46–47° C., $[\alpha]_D$ +12° (*c*, 1.1 in chloroform). Calc. for $C_{20}H_{38}O_4$: C, 70.13; H, 11.18; $(CH_3)_2C$, 12.29%. Found: C, 69.85; H, 11.28; $(CH_3)_2C$, 12.3%.

Diisopropylideneustilate B

After two recrystallizations from methanol, the isopropylidene derivative from the third fraction of the above distillation melted at 42.5–43° C. with $[\alpha]_D$ +18° (*c*, 1.0 in chloroform). Calc. for $C_{22}H_{40}O_5$: C, 68.71; H, 10.49%. Found: C, 68.27; H, 10.48%. Acid hydrolysis liberated acetone equivalent to an isopropylidene group content of 22.0%. Calc. for a diisopropylidene derivative 21.9%. Alkaline hydrolysis liberated acetone equivalent to an isopropylidene group content of 12.3%. Calc. for one alkali-labile isopropylidene group, 10.94%. Absorption characteristic of hydroxyl groups was not present in its infrared spectrum. The substance possessed a strong absorption band at 5.54μ, a considerably shorter wave length than is found for the carbonyl group of acyclic esters of carboxylic acids. For example, methyl isopropylideneustilate A possessed a carbonyl absorption band at 5.88μ.

Methyl Ustilate A

The methyl isopropylideneustilate A, 600 mgm., was treated with 15 ml. of boiling 2% methanolic hydrogen chloride for two hours. The colorless solution was poured into water and the precipitate was extracted with chloroform. The dried chloroform extract was evaporated *in vacuo* to a crystalline solid which, after two recrystallizations from methanol, melted at 85.5–86° C. with $[\alpha]_D$ $-7°$ (*c*, 1 in methanol). Calc. for $C_{17}H_{34}O_4$: C, 67.53; H, 11.33%. Found: C, 67.94; H, 11.38%.

Ustilic Acid A

Alkaline hydrolysis of the above described methyl ustilate A yielded a substance, m.p. 112–113° C., $[\alpha]_D$ $-8°$ (*c*, 1 in methanol). Calc. for $C_{16}H_{32}O_4$: C, 66.64; H, 11.19%; neutral equiv. 288.4. Found: C, 66.92; H, 11.22%; neutral equiv. 288. Its infrared absorption spectrum is shown in Fig. 1.

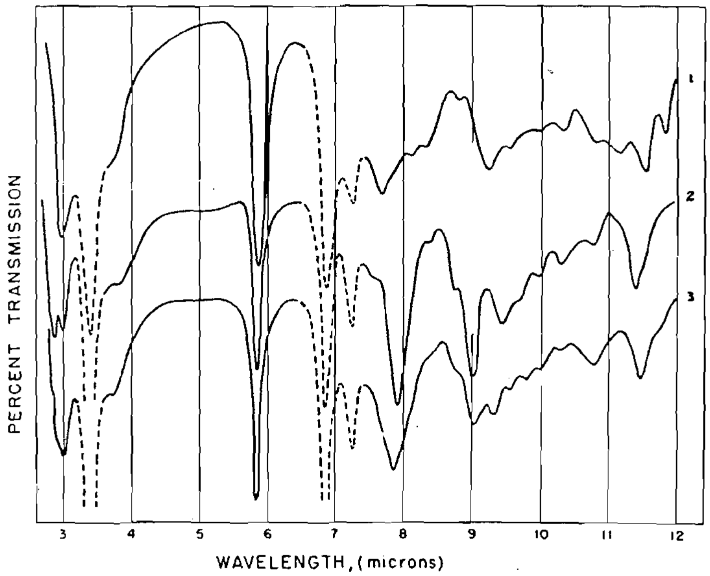


FIG. 1. Infrared spectra. Curve 1, ustilic acid A; Curve 2, ustilic acid B; Curve 3, ustilic acid mixture. – – – – Nujol absorption bands.

Methyl Ustilate B

The diisopropylideneustilate B, 2 gm., was treated with 25 ml. of boiling 2% methanolic hydrogen chloride for two hours. The resulting yellow-colored solution was poured into water, the mixture was extracted with chloroform, and the chloroform extract was washed with water and dried by filtration. The chloroform was removed *in vacuo* to yield a crystalline material which, after three recrystallizations from methanol, melted at 74–75° C. with $[\alpha]_D$ $-8.5°$ (*c*, 1.0 in methanol). Calc. for $C_{17}H_{34}O_5$: C, 64.13; H, 10.76%. Found: C, 64.55; H, 10.91%.

Ustilic Acid B

Alkaline hydrolysis of the above described methyl ustilate B yielded a material, m.p. 140–141° C., $[\alpha]_D$ $-10°$ (*c*, 1.0 in methanol). Calc. for $C_{16}H_{32}O_5$: C, 63.14; H, 10.60%; neutral equiv. 304.4. Found: C, 63.09, 63.27; H, 10.62, 10.27%; neutral equiv. 305. Its infrared absorption spectrum is shown in Fig. 1.

Separation of Ustilic Acid Derivatives by Extraction

A ustilic acid mixture, m.p. 123–128° C., 47.3 gm., was dissolved by being shaken in 500 ml. of dry acetone which contained 5 ml. of concentrated sulphuric acid. The solution was allowed to stand at room temperature for five hours. After this time, the solution was diluted with 800 ml. of Skellysolve C and 800 ml. of ice-cold water was added. The mixture was shaken vigorously for 30 sec., the layers were allowed to separate, and the bottom aqueous layer was withdrawn and discarded. The hydrocarbon layer was washed three times with 200 ml. amounts of water to remove the sulphuric acid and most of the acetone. A cold solution of 20 gm. of potassium hydroxide in 130 ml. of water was then added and the mixture was shaken vigorously for one minute. On the addition of 150 ml. of 50% aqueous ethanol, the emulsion broke and the bottom aqueous layer was separated. The Skellysolve C solution was washed twice with 100 ml. amounts of the 50% ethanol and these extracts were combined with the main aqueous extract. The combined aqueous extracts were extracted with 200 ml. of Skellysolve C and this extract was combined with the main Skellysolve C solution. Chloroform, 150 ml., was added to the combined aqueous layers and this mixture was acidified by the addition of 200 ml. of 2 *N* sulphuric acid. The chloroform layer was washed three times with 100 ml. volumes of water, dried over sodium sulphate and evaporated to a yellow oil which soon crystallized. The yield was 34.8 gm. of crude monoisopropylideneustilic acid A which on hydrolysis gave essentially pure ustilic acid A. The Skellysolve C solution was washed with 50 ml. amounts of water until neutral and dried over sodium sulphate. Evaporation gave 15.1 gm. of a crystalline material which after recrystallization from 25 ml. of methanol melted at 42–42.5° C. Pure diisopropylideneustilate B melted at 42.5–43° C. Hydrolysis yielded essentially pure ustilic acid B.

Palmitic Acid

Methyl ustilate A, 500 mgm., was treated with 10 ml. of boiling 47% hydriodic acid and 2 gm. of red phosphorus for four hours. The reaction mixture was poured into water and the aqueous mixture was extracted with chloroform. The chloroform extract was evaporated to a partially crystalline oil which was dissolved in a mixture of 25 ml. of ethanol, 4 ml. of 10% sodium hydroxide, and 2 ml. of settled Raney nickel catalyst. The mixture was stirred in a hydrogen atmosphere at slightly positive pressure for one hour. The catalyst was removed by filtration and washed several times with boiling water. The combined alcoholic and aqueous filtrates were evaporated to about 75 ml. and the solution was made strongly acid with sulphuric acid. The colorless precipitate was collected by filtration and washed with water. After three recrystallizations from ethanol, the material melted at 59–61° C. and this melting point was not depressed on admixture with an authentic sample of palmitic acid, m.p. 61.5–62° C.

Periodate Oxidation of the Ustilic Acids and Determination of the Formaldehyde Formed

The ustilic acid, 100 mgm., was dissolved in 5 ml. of 0.1 *N* sodium hydroxide and the solution was made neutral to phenolphthalein with 0.1 *N* sulphuric acid. After the addition of 25 ml. of 0.1 *N* sodium periodate and 25 ml. of water, the mixture was distilled at atmospheric pressure to about 25 ml. volume. In the case of the ustilic acid B the distillate contained a small amount of a steam-distillable, water-insoluble solid which was removed by filtration. In each case, on adding the clear filtrate to 100 ml. of a buffered dimedon solution (25) a precipitate formed which, after isolation and recrystallization from ethanol, possessed, and did not depress, the melting point of the methone derivative of formaldehyde.

A weighed amount of the ustilic acid, 15 to 20 mgm., was dissolved in 20 ml. of 0.1 *N* sodium hydroxide by heating on the steam bath and the cooled solution was diluted to 100 ml. Three 25 ml. aliquots were transferred to 100 ml. flasks and 5 ml. volumes of 0.1 *M* periodic acid were added to each flask. After one, two and four hours, 10 ml. of *N* sulphuric acid and 5 ml. of *M* sodium arsenite solution were added to a flask and the solution was diluted to 100 ml. One milliliter aliquots were transferred to test tubes for development of the chromotropic acid–formaldehyde color reaction in the manner described by Lambert and Neish (14). A standard curve was established by oxidizing, under the above conditions, 5, 10, and 15 ml. volumes of a standard 0.002 *M* solution of mannitol. A reagent blank was used to set the spectrometer at 100% transmittance. The results obtained with the ustilic acids A and B and the above described crude and purified ustilic acid mixtures are shown in Table II.

Rates of Oxidation of Methyl Ustilates by Chromic Oxide

Chromic oxide, 1.00 gm., was dissolved in 2 ml. of water and the solution was diluted to 250 ml. with pure acetic acid. The methyl ustilate, 80–85 mgm., was dissolved in 40 ml. of acetic acid and, at zero time, 50 ml. of the chromic oxide solution was added. The resulting solution was diluted to 100 ml. with acetic acid and, at the various times, 10 ml. aliquots were added to 50 ml. of 0.2 *N* sulphuric acid followed by the addition of about 0.5 gm. of potassium iodide. The liberated iodine was determined by titration with standard sodium thiosulphate solution to the starch end point. The difference in titer between the oxidation mixture and a reagent blank was taken as equivalent to the oxidant consumed by the methyl ustilate. The data plotted in Fig. 2 show that the methyl ustilates A and B rapidly consumed oxidant equivalent to four and five atoms of oxygen, respectively, and that, on this basis, the above described purified methyl ustilate mixture contained about 70% methyl ustilate A and 30% methyl ustilate B.

Pentadecanedioic Acid

Methyl ustilate A, 200 mgm. (0.662 mM.), was dissolved in 5 ml. of glacial acetic acid and 5.30 me. of chromic oxide in 6 ml. of acetic acid was added. After 24 hr. at room temperature, the solution was diluted with 100 ml. of water and the precipitate which formed was gathered by filtration and washed with water. The material was dissolved in 10 ml. of 0.5 *N* sodium hydroxide solution and the mixture was heated on the steam bath for 0.5 hr. The solution

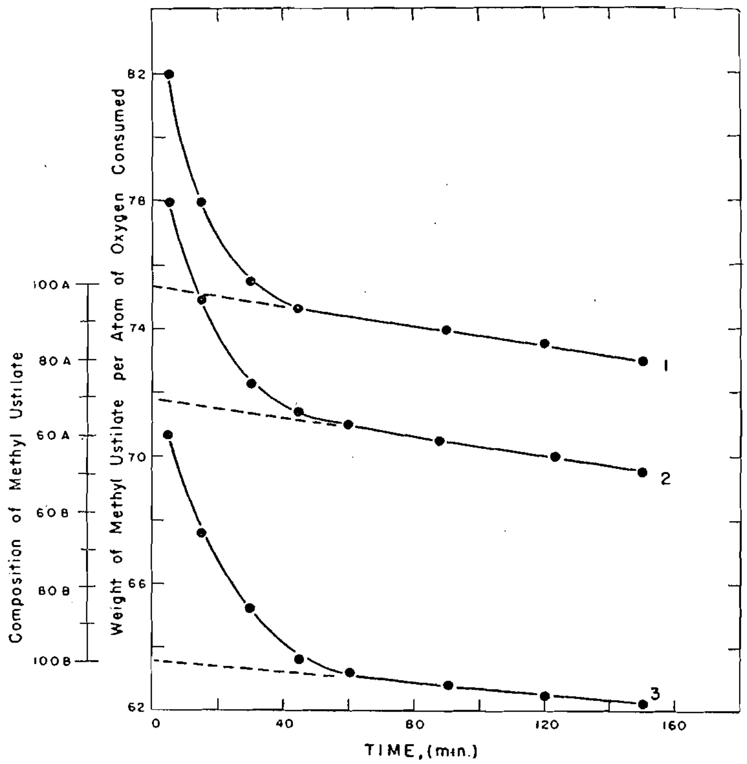


FIG. 2. Oxidation with chromic oxide in acetic acid of methyl ustilate A (Curve 1), methyl ustilate mixture (Curve 2), and methyl ustilate B (Curve 3).

was filtered and acidified with 10 ml. of *N* hydrochloric acid. The precipitate was collected, washed with water, dried, and recrystallized four times from ethyl acetate to yield 71 mgm. of material, m.p. 113–114° C. The melting point recorded for pentadecanedioic acid is 114.6–114.8° C. (4). Calc. for $C_{13}H_{26}(COOH)_2$: C, 66.15; H, 10.36%; neutral equiv., 136.2. Found: C, 66.12; H, 10.36%; neutral equiv., 136.

Methyl 2-Keto-15-carboxypentadecanoate

Chromic oxide, 5.00 gm., in 10 ml. of water was added to 50 ml. of acetic acid and the resulting solution was added to a stirred solution of 3.20 gm. of methyl ustilate B in 50 ml. of acetic acid kept at 0° C. The mixture soon became homogeneous and the solution was kept at 4° C. for 25 hr. The precipitate which had formed on the addition of 250 ml. of water and cooling to 4° C. for three hours was collected and washed with water. The yield was 2.61 gm. of a greenish powder which was extracted with 100 ml. of boiling Skellysolve C. On cooling the extract, 1.96 gm. of a white crystalline solid, m.p. 71–74° C., was deposited. Recrystallization from two parts acetone gave 1.74 gm., m.p. 84.5–86°C. Three further recrystallizations from acetone gave a nicely crystalline product, m.p. 87–87.5° C. This ester, like other α-keto esters, was highly susceptible to alkaline hydrolysis and it was not possible to determine the neutral equivalent by direct titration. Titration of an alcoholic solution with 0.1 *N* aqueous sodium hydroxide to phenolphthalein indicator gave a combined neutral and saponification equivalent of 150.5. Calculated, 150.2. Calc. for $C_{16}H_{28}O_5$: C, 63.98; H, 9.39%. Found: C, 64.13; H, 9.55%.

Tetradecanedioic Acid

The monomethyl ester of 2-ketopentadecanedioic acid, 230 mgm., was hydrolyzed to yield 217 mgm. of the 2-ketopentadecanedioic acid as a white powder which was not purified. The material, 200 mgm., was stirred at room temperature with 10 ml. of 30% hydrogen peroxide (13) for 20 hr. The resulting material was washed with water and air-dried. After recrystallization, first from 70% ethanol, then twice from ethyl acetate, and once more from 70% ethanol, the yield was 68 mgm., m.p. 124.5–125° C. Tetradecanedioic acid is reported (4) to melt at 125.8° C. Calc. for $C_{14}H_{26}O_4$: C, 65.06; H, 10.14%; neutral equiv., 129.2. Found: C, 65.20; H, 10.11%; neutral equiv., 129.

1,14-Dihydroxytetradecane

Ustilic acid B, 3.18 gm. (10.5 mM.), was suspended in 45 ml. of acetic acid and the stirred mixture was heated to 45° C. Commercial sodium bismuthate (28), about 80% pure, 7.50 gm. (about 21.5 mM.), was added over a period of 20 min. and the reaction mixture, kept in a nitrogen atmosphere, was stirred for a total of 40 min. Benzene, 50 ml., and water, 45 ml., were added and, after vigorous shaking, the aqueous layer was withdrawn and re-extracted with 50 ml. of benzene. The benzene extracts were washed with 20 ml. of 50% aqueous acetic acid, combined, and washed free of acetic acid with water. After it was dried over sodium sulphate the solution was filtered and evaporated *in vacuo* to an oil which hardened at room temperature. The material, which was extremely prone to auto-oxidation, was dissolved in 50 ml. of ethanol and hydrogenated at 1500 p.s.i. for four hours at 100° C. using Raney nickel catalyst. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to a crystalline solid which was triturated with low-boiling petroleum ether to yield 2.19 gm. of a faintly greenish-tinted material, m.p. 78–82° C. Distillation at 10^{-2} mm. pressure gave 1.81 gm., m.p. 83–85° C., of a substance which melted at 85–86° C. after one recrystallization from benzene. Chuit (4) has reported 1,14-dihydroxytetradecane to melt at 84.8° C. Calc. for $C_{14}H_{30}O_2$: C, 72.96; H, 13.12. Found: C, 72.95; H, 13.08. The crude yield was 91%.

Lead Tetraacetate Oxidations

About 0.25 mM. of the compound was dissolved in 5 ml. of acetic acid, 15 ml. of 0.13 *N* lead tetraacetate in glacial acetic acid solution was added, and the solution was diluted to 25 ml. After one and two hours, the excess oxidant in 10 ml. aliquots was determined in the usual manner (9). Both methyl ustilate A and methyl ustilate B consumed one mole of oxidant per mole of ester.

15-Hydroxypentadecanoic Acid

Methyl ustilate A, m.p. 84–85° C., 4.19 gm., was dissolved in 80 ml. of acetic acid and 8 gm. of lead tetraacetate was added. The reaction mixture was shaken until homogeneous and left at room temperature for one hour. Benzene, 50 ml., was added and the mixture was poured into 200 ml. of water which contained a little glycerol to destroy the excess oxidant. After vigorous shaking, the water layer was withdrawn and re-extracted with 25 ml. of benzene. The combined benzene extracts were washed with water until free of acetic acid and evaporated to an oil which soon crystallized. This substance, methyl aldehydopentadecanoate, could not be obtained in a highly pure condition because of its strong tendency to undergo auto-oxidation and was hydrogenated directly at 1500 p.s.i. for two hours at 100° C. over Raney nickel catalyst in 75 ml. of ethanol. The catalyst was removed by filtration and the ethanol solution was evaporated to yield 3.70 gm., 98% yield, of a crystalline solid, m.p. 48–51° C. Recrystallization from one part methanol gave pure methyl 15-hydroxypentadecanoate, m.p. 51.5–52° C. Calc. for $C_{16}H_{32}O_3$: C, 70.56; H, 11.84%. Found: C, 70.11; H, 11.63%. Chuit and Hausser (5) reported this substance to melt at 52–52.5° C. Alkaline hydrolysis of this ester gave an acid which after three recrystallizations from ether melted at 84.5–85° C. 15-Hydroxypentadecanoic acid is reported (5) to melt at 84° C. Calc. for $C_{15}H_{30}O_3$: C, 69.74; H, 11.70%; neutral equiv., 258.4. Found: C, 69.66; H, 11.97%; neutral equiv., 259.

Methyl 2D,15-Dihydroxypentadecanoate

Methyl ustilate B, m.p. 73–74° C., 1.92 gm., was dissolved in 60 ml. of acetic acid and 3.2 gm. of lead tetraacetate was added. The mixture was shaken until homogeneous and left at room temperature for one hour. The methyl 2-hydroxyaldehydopentadecanoic acid which formed was isolated as described above. The crude crystalline product was hydrogenated at 1500 p.s.i. for three hours at 100° C. over Raney nickel catalyst in 25 ml. of ethanol. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to a crystalline solid, 1.57 gm., 90% yield, m.p. 68–72° C. Several recrystallizations from two parts methanol gave pure methyl 2D,15-dihydroxypentadecanoate, m.p. 74.3–75.3° C. Calc. for $C_{16}H_{32}O_4$: C, 66.64; H, 11.19%. Found: C, 66.80; H, 10.96%.

2D,15-Dihydroxypentadecanoic Acid

Alkaline hydrolysis of the above described ester gave a substance which melted at 102–103° C. after three recrystallizations from methanol and which was very weakly levorotatory in methanol. Calc. for $C_{15}H_{30}O_4$: neutral equiv., 274.4. Found: neutral equiv., 275.

1,2-Isopropylidene-2D,15-dihydroxypentadecanoate

The dihydroxyacid, 80 mgm., was treated with 5 ml. of dry acetone and two drops of sulphuric acid for 20 hr. at room temperature. The product, isolated in the usual manner, was recrystallized from ligroin to yield 62 mgm. of material, m.p. 49–50° C., with $[\alpha]_D$ +5° (*c*, 0.8 in chloroform). Calc. for $C_{18}H_{34}O_4$: C, 68.75; H, 10.90%. Found: C, 69.43; H, 11.06%.

2D-Hydroxypentadecanoic Acid

Crude methyl 2D-hydroxyaldehydopentadecanoate, prepared as described above from 1.90 gm. of methyl ustilate B, was dissolved at 0° C. in 10 gm. of ethyl mercaptan which contained 1.0 gm. of anhydrous zinc chloride (30). The solution was kept at 4° C. for four hours and poured into 100 ml. of aqueous sodium bicarbonate solution. The precipitate, collected by filtration, was extracted three times with boiling chloroform and the combined chloroform extracts were used to extract the aqueous phase. The chloroform solution was dried over sodium sulphate and evaporated *in vacuo* to an oil, which was dried *in vacuo* at 60° C. Calc. for $C_{20}H_{40}O_3S_2$: C, 61.18; H, 10.27%. Found: C, 60.99; H, 10.46%. The substance, n_D^{25} 1.4890, was dissolved in 50 ml. of ethanol containing 40 ml. of settled Raney nickel, and the mixture was refluxed for 2.5 hr. The catalyst was collected by filtration and twice extracted with 50-ml. amounts of ethanol. The combined ethanolic solutions were evaporated *in vacuo* to an oil which crystallized at room temperature. The substance was distilled to yield a 1.21 gm. fraction, b.p. 117–118° C. at about 0.05 mm., which melted at 23–29° C. Alkaline hydrolysis of the material, 1.12 gm., saponification equivalent 260, gave an acidic product which was dissolved in 5 ml. of chloroform and petroleum ether was added to turbidity. On cooling, there crystallized 0.99 gm. of a substance, m.p. 88–89° C. Three recrystallizations from three parts 90% methanol gave a substance, m.p. 89.5–90° C., $[\alpha]_D$ −0.8° (*c*, 5 in ethanol). Calc. for $C_{15}H_{30}O_3$: C, 69.74; H, 11.70%; neutral equiv., 258.4. Found: C, 69.39; H, 11.12%; neutral equiv., 258.

*Sodium 2*D*-hydroxypentadecanoate*

2D-Hydroxypentadecanoic acid, 130 mgm., was dissolved in 15 ml. of ethanol and the solution was neutralized to phenolphthalein with 0.1 *N* sodium hydroxide solution. Evaporation gave a solid which was recrystallized first from 50% ethanol then from pure ethanol to yield 101 mgm. of a nicely crystalline product, $[\alpha]_D$ +15° (*c*, 1 in 50% aqueous ethanol). Calc. for $C_{15}H_{29}O_3Na$: C, 64.26; H, 10.43%. Found: C, 64.11; H, 10.23%.

*Sodium 2*L*-Hydroxypentanoate*

The previously described (18) methyl 2L-hydroxypentanoate, $[\alpha]_D$ +16.6° (*c*, 1.2 in chloroform), 446 mgm., was heated on the steam bath for one hour with 35 ml. of 0.1 *N* sodium hydroxide. The neutral solution was evaporated to a crystalline solid which was dissolved in 20 ml. of ethanol. On the addition of 40 ml. of dry acetone and cooling to −8° C., there crystallized 300 mgm. of the salt in a nicely crystalline condition. The substance was not very hygroscopic. Calc. for $C_5H_9O_3Na$: H, 6.47%. Found: H, 6.54%. The rotations for this substance are given in Table I.

*15*D*,16-Dihydroxyhexadecanohydrazide* (*Ustilohydrazide A*)

Methyl ustilate A, 0.5 gm., was heated under reflux for 30 min. with 0.5 ml. of hydrazine hydrate and 1 ml. of ethanol. Four milliliters of ethanol was added and the heating was continued for 30 min. On cooling, a near quantitative yield of the hydrazide crystallized. After recrystallization from ethanol the substance melted at 140.5–141.5° C. Calc. for $C_{16}H_{34}O_3N_2$: C, 63.54; H, 11.33%. Found: C, 63.59; H, 10.74%.

*2*D*,15*D*,16-Trihydroxyhexadecanohydrazide* (*Ustilohydrazide B*)

This substance, prepared as described above and purified by recrystallization from ethanol, melted at 164–165° C. Calc. for $C_{16}H_{34}O_4N_2$: C, 60.34; H, 10.76%. Found: C, 60.27; H, 10.68%.

*2*D,*15-Dihydroxypentadecanohydrazide*

This substance, prepared as described above and purified by recrystallization from ethanol, melted at 129–130° C. with $[\alpha]_D$ +24° (*c*, 0.2 in 50% aqueous ethanol) measured in a 4 dm. tube. Calc. for $C_{15}H_{32}O_3N_2$: C, 63.14; H, 11.19%. Found: C, 63.28; H, 11.22%.

15-Hydroxypentadecanohydrazide

This substance, prepared as described above and purified by recrystallization from ethanol, melted at 142.5–143.5° C. Calc. for $C_{15}H_{32}O_2N_2$: C, 66.15; H, 11.84%. Found: C, 66.06; H, 12.18%.

Meso-1,2,15,16-tetrahydroxyhexadecane

Diisopropylidene ustilate B, m.p. 42.5–43° C., 3.84 gm. (10 mM.), was dissolved in 50 ml. of dry ether and an excess of an ethereal solution of lithium aluminum hydride was added. After the solution had been allowed to stand at room temperature for two hours, it was poured into ice-water and acidified with 10% sulphuric acid. The resulting mixture was extracted twice with chloroform and the filtered extract was evaporated to a crystalline solid. The material was dissolved in 25 ml. of ethanol and 10 ml. of 0.1 *N* hydrochloric acid was added. After the solution had been boiled for 15 min., 20 ml. of water was added and the solution was kept at 4° C. for two hours. The crystalline precipitate, 2.89 gm., 95% yield, melted at 127–130° C. After one recrystallization from methanol, the yield was 2.70 gm., m.p. 132–134° C. Further recrystallizations from methanol or acetic acid did not raise the melting point above 133–134° C. The material was insoluble in ethyl acetate, chloroform, ether, or water and only sparingly soluble in cold pyridine, acetic acid, methanol, or ethanol. The substance appeared to possess a slight optical activity, $[\alpha]_D^{40} < -0.5°$ (*c*, 1 in methanol). Calc. for $C_{16}H_{34}O_4$: C, 66.18; H, 11.80%. Found: C, 66.15; H, 11.46%. Oxidation with an excess of lead tetraacetate in acetic acid resulted in the consumption of 2.0 moles of the oxidant after one- and two-hour reaction times.

Diisopropylidene-meso-1,2,15,16-tetrahydroxyhexadecane

The 1,2,15,16-tetrahydroxyhexadecane, m.p. 133–134° C., 500 mgm., was suspended in 20 ml. of dry acetone which contained two drops of sulphuric acid. After the mixture had been shaken for one hour, solution was complete. The mixture was left at room temperature overnight and poured into 100 ml. of aqueous bicarbonate solution. The material, which crystallized immediately, was gathered by filtration and washed with water. The yield was 630 mgm., 99%, m.p. 65–66° C. Recrystallization from methanol gave a pure product, m.p. 66.5–67° C., which was optically inactive (*c*, 1.5 in chloroform). Calc. for $C_{22}H_{42}O_4$: C, 71.29; H, 11.42%. Found: C, 70.98; H, 11.81%.

Meso-1,2,15,16-tetraacetoxyhexadecane

Meso-1,2,15,16-tetrahydroxyhexadecane, 240 mgm., was heated on the steam bath for one hour with 10 ml. of acetic anhydride and 200 mgm. of sodium acetate. The mixture was stirred overnight with 100 ml. of water. The crystalline precipitate which had formed was washed with water and dried. The yield was 380 mgm., 100%, m.p. 79–80° C. Recrystallization from methanol gave coarse crystals, m.p. 79.5–79.7° C., of an optically inactive substance (*c*, 1.5 in chloroform). Calc. for $C_{24}H_{42}O_8$: C, 62.87; H, 9.23%. Found: C, 62.87; H, 9.15%.

1,2-Monoisopropylidene-1,2L,16-trihydroxyhexadecane

Methyl isopropylideneustilate A, 1 gm., was dissolved in 25 ml. of ether and the solution was treated with an excess of an ethereal solution of lithium aluminum hydride. After 0.5 hr. at room temperature, the solution was poured into 50 ml. of ice-water which contained 2 ml. of acetic acid and the mixture was extracted several times with ether. The ether extracts were combined, washed with water, dried over sodium sulphate, and evaporated to a crystalline solid. Recrystallization from ligroin gave 590 mgm. of a substance, m.p. 60–61° C., $[\alpha]_D$ +7.5° (*c*, 1 in benzene) and +12° (*c*, 1 in chloroform). Calc. for $C_{19}H_{38}O_3$: C, 72.49; H, 12.18%. Found: C, 72.42; H, 12.05%.

1,2L,16-Trihydroxyhexadecane

Acid hydrolysis of the above described isopropylidene derivative, 225 mgm., yielded 190 mgm. of product, m.p. 107.5–108.5° C. After two recrystallizations from methanol, the material melted at 108.5–109° C. with $[\alpha]_D$ −7° (*c*, 1 in methanol). Calc. for $C_{16}H_{34}O_3$: C, 70.04; H, 12.49%. Found: C, 70.52; H, 12.28%.

1,2L,16-Triacetoxyhexadecane

Acetylation of the above described 1,2L,16-trihydroxyhexadecane, 80 mgm., with acetic anhydride and sodium acetate and isolation of the product which crystallized on decomposing the reaction mixture with water gave 105 mgm., 90% yield, of a low melting compound. After recrystallization from 30–40 petroleum ether, the material melted at 24–25° C. with $[\alpha]_D$ −0.8° (*c*, 1 in chloroform). Calc. for $C_{22}H_{40}O_6$: C, 65.97; H, 10.07%. Found: C, 66.19; H, 10.20%.

Conversion of Ustilic Acid B to Ustilic Acid A

Diisopropylidene ustilate B, 2.56 gm., was dissolved in 20 ml. of ethanol and 5 ml. of 2 *N* potassium hydroxide was added. The solution was refluxed for 30 min., diluted with 40 ml. of water, 35 ml. of chloroform, and 15 ml. of *N* hydrochloric acid. After shaking, the chloroform layer was isolated and washed twice with water, dried over sodium sulphate, and evaporated to a crystalline solid. The material was treated with an excess of diazomethane in ether, the ether was removed by evaporation, and the residue was dissolved in 5 ml. of methanol. The solution was filtered and diluted with water to turbidity. On cooling there crystallized 2.19 gm., 92%, of methyl 15,16-isopropylideneustilate B, m.p. 42–43° C. After recrystallization from ligroin, the material melted at 42.5–43° C. with $[\alpha]_D$ +6° (*c*, 1 in chloroform). Calc. for $C_{20}H_{38}O_5$: C, 67.00; H, 10.68%. Found: C, 67.38; H, 10.50%. This material, 1.68 gm., was dissolved in 10 ml. of dry pyridine which contained 1.60 gm. of *p*-toluenesulphonyl chloride and the solution was left at room temperature for two days. The mixture was stirred with 50 ml. of 50% aqueous acetone for 30 min. and extracted with chloroform. The chloroform extract was washed several times with water and evaporated to an oil which did not crystallize. The oil was dissolved in 25 ml. of dry acetone which contained 10 gm. of sodium iodide and the mixture was heated at 140° C. for five hours. The reaction mixture was poured into 50 ml. of water and the free iodine was reacted with sodium thiosulphate. Extraction with chloroform gave a brown-colored oily product which gave a strong test for iodine. The substance was dissolved in 25 ml. of ethanol containing 10 ml. of 2 N aqueous sodium hydroxide and about 2 ml. of Raney nickel catalyst. The solution was hydrogenated for two hours at 1000 p.s.i. pressure. After removal of the catalyst, the solution was concentrated to about 10 ml., acidified with hydrochloric acid to Congo red indicator, and the solid precipitate was washed with water, dried at 65° C. *in vacuo*, and dissolved in 25 ml. of dry acetone which contained four drops of concentrated sulphuric acid. The solution was left at room temperature for seven hours, diluted with 50 ml. of chloroform, and the mixture poured into 50 ml. of water. After shaking, the chloroform layer was isolated and washed several times with water, dried over sodium sulphate, and evaporated *in vacuo* to yield a brown-colored oil which was treated with an excess of diazomethane in ether. Solvent removal gave an oil which was distilled at about 0.1 mm. to yield a 463 mgm. fraction, b.p. 140–143° C. The substance, $[\alpha]_D$ +13° (c, 1 in chloroform), melted at 46–47° C. and a mixed melting point with methyl isopropylideneustilate A, m.p. 47–47.5° C., was 46–47.5° C. The over-all yield from methyl 15,16-isopropylideneustilate B was 29%. Methanolysis to remove the isopropylidene group gave methyl ustilate A, m.p. 85–86° C.

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