bond, one split and the other not, 4.05 (d, 2, RR'C=CHCH$_2$OH, J = 7.0 and shifted downfield by addition of TCAIC, 5.53 (t, 1, RR'C=CHCH$_2$OH, J = 7.0); mass spectrum (70 eV) m/e (rel intensity) 154 (7), 136 (40), 121 (48), 107 (25), 93 (63), 79 (53), 69 (100), 55 (35), 41 (88).

Anal. Calcd for $C_{10}H_{18}O$: C, 77.86; H, 11.76. Found: C, 77.81; H, 11.79.

E-3,3-Dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol (21).—The methyl ester of the *E* unsaturated acid was reduced in the same way as the *Z* ester to the *E* alcohol and purified by glpc. Compound **21** had the following nmr spectrum: 0.95 (s, 6, geminal CH$_3$), 1.4–1.7 (m, 4, two CH$_2$), 1.96 (s, 2, CH$_2$ trans to the carbinol group), 2.18 (s, 1, OH), 2.20 2, (t, 2, CH$_2$ cis to the carbinol group), 4.13 (d, 2, CH$_2$OH), 5.37 1, (t, 1, C=CH). The ir spectrum was similar to that of **2**.

Anal. Calcd for $C_{10}H_{18}O$: C, 77.86; H, 11.76. Found: C, 77.64; H, 11.83.

Z-3,3-Dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde (3).—Active MnO$_2$ was prepared as described by Attenburrow, *et al.*[24] Stereospecific oxidation of the *Z* alcohol **2** (100 mg) by stirring with 3.3 g of active MnO$_2$ in 30 ml of pentane for 30 min at 0° produced the *Z*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde in quantitative yield.[17] The reaction mixture was filtered, the pentane removed by evaporation, and the aldehyde purified by glpc. Chromatographically pure samples were identical with natural compound **3** in glpc behavior, mass spectrum, and biological activity.

(24) J. A. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker, *J. Chem. Soc.*, 1094 (1952).

Compound **3** had the following spectral characteristics: nmr spectrum 0.93 (s, 6, geminal CH$_3$), 1.2–2.0 (broad m, 4, two CH$_2$), 2.17 (t, 2, CH$_2$ trans to aldehyde group), 2.42 (s, 2, CH$_2$ cis to aldehyde group), 5.74 (d, 1, CHO); mass spectrum (70 eV) m/e (rel intensity) 152 (34), 137 (90), 109 (45), 95 (28), 81 (45), 69 (59), 55 (30), 53 (30), 41 (100).

E-3,3-Dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde (4).—The *E* alcohol **21** was oxidized with active MnO$_2$ to the *E* aldehyde in the same way as the *Z* alcohol. *E*-3,3-Dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde was found to be identical with natural compound **4** in glpc behavior, mass spectrum, and biological activity.

Compound **4** had the following spectral characteristics: nmr spectrum 0.89 (s, 6, geminal CH$_3$), 1.2–1.9 (m, 4, two CH$_2$), 2.00 (s, 2, CH$_2$ trans to aldehyde group), 2.61 (t, 2, CH$_2$ cis to aldehyde group), 5.60 (d, 1, C=CH), 9.78 (d, 1, CHO); mass spectrum (70 eV) m/e (rel intensity) 152 (46), 137 (46), 119 (24), 109 (63), 93 (29), 81 (38), 69 (63), 55 (33), 41 (100).

Registry No.—**1**, 26532-22-9; **2**, 26532-23-0; **3**, 26532-24-1; **4**, 26532-25-2; **5**, 30346-11-3; **6**, 30346-12-4; **7**, 30346-13-5; **9**, 30346-14-6; **11**, 30346-15-7; **12**, 6090-09-1; **13**, 30346-17-9; **14**, 30346-18-0; **15**, 30346-19-1; **16**, 30346-20-4; **17**, 30346-21-5; **18**, 30346-22-6; **19**, 30346-23-7; **19** free acid, 30346-24-8; **20**, 30346-25-9; **20** free acid, 30346-26-0; **21**, 30346-27-1.

Identification of Two Conjugated Pentaenoic Acids in the Insect Fat, Aje

JAMES CASON,* ROMAN DAVIS, AND MICHAEL H. SHEEHAN

Chemical Laboratories, University of California, Berkeley, California 94720

Received February 9, 1971

The fatty acids from aje, body fat of the coccid *Llaveia axin*, have been examined. In addition to the normal saturated acids at the C_{14}, C_{18}, and C_{20} molecular weights, and the unsaturated C_{18} acids, oleic and linoleic, there were present pentaunsaturated acids at the C_{12} and C_{14} levels. The latter components were so unstable that separation in a pure condition was not feasible; however, the ultraviolet spectrum of the mixture of acids was virtually identical with that which has been reported for a pentaenoic fatty acid after alkali isomerization to a conjugated system. The conjugated system in both the C_{12} and C_{14} acids was shown to be in a terminal position by identification of formaldehyde after ozonolysis. The appropriate fragment from ozonolysis also established the other end of the conjugated system as at carbon-3 in the C_{12} acid and at carbon-5 in the C_{14} acid. Confirmatory evidence was obtained from mass spectrometry of the deuterated esters. Thus, assigned structures for the conjugated pentaenoic acids, believed to be the first found in natural products, are 3,5,7,9,11-dodecapentaenoic acid and 5,7,9,11,13-tetradecapentaenoic acid. The names C_{12}-ajenoic acid and C_{14}-ajenoic acid are proposed.

The body fat of the Mexican and Central American scale insect *Llaveia axin*, is known as aje. It is in current use in the villages, both as an unguent and a drying oil in gourd painting.[1] References to the substance extend at least as far back as the sixteenth century; however, there has been no significant chemical investigation of the material. Although samples of the solid fat form a crust on the surface relatively quickly, and a major use has been as a vehicle for pigments in gourd painting, the iodine number has been reported[2] considerably lower than expected for a drying oil. The present investigation has been directed toward examination of the fatty acids in aje.

Aje was found to contain no significant amounts of free fatty acids. Acids released by saponification gave only 50–60% yields of methyl ester on acid-catalyzed esterification, with extensive polymer formation; however, base-catalyzed esterification[3] of freshly prepared acids gave 75–85% yields of methyl ester. On standing at room temperature in air or under nitrogen, in solution or neat, the ester exhibited formation of a polymeric oil within a few hours. Gas chromatography of the esters on silicone and on DEGS (diethylene glycol succinate) revealed the presence of significant amounts of only four components, whose retention times corresponded precisely with methyl stearate (representing 51% of total area under the four peaks), oleate (18.5%), linoleate (15.5%), and eicosanoate (15%). Since a mixture of this composition would not give a rapid polymerization, it was suspected that one or more of the peaks would prove not to contain the common ester with that retention time. However, when the component responsible for each peak was collected and identified, each proved to be the well-known substance with the observed retention time.

(1) A historical survey, as well as description of current use of aje, have been reported by Mrs. Katharine D. Jenkins in the Actas y Memorias of the 35th International Congress of Americanists, Mexico, 1964, pp 625–636. We are greatly indebted to Mrs. Jenkins for the samples of aje utilized in the current investigation.

(2) Francisco Giral, Mexico City, in a private communication to Mrs. Jenkins, dated Aug 30, 1963, reported that he found iodine numbers in the range 74–84.

(3) F. H. Stodola, *J. Org. Chem.*, **29**, 2490 (1964).

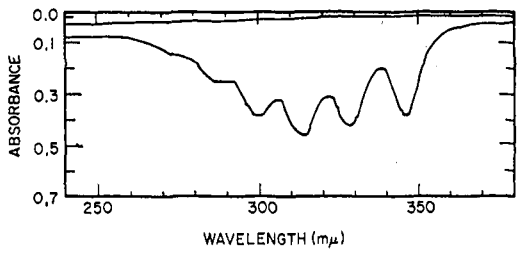


Figure 1.—Ultraviolet absorption of 0.008 *M* solution in methanol of freshly prepared aje acid methyl esters: 0.05-cm cell length; Perkin-Elmer Model 202 uv spectrophotometer. Upper tracing is solvent *vs.* solvent. Prompt hydrogenation of this sample of esters and analysis by gas chromatography indicated about 21% (molar) total of highly unsaturated C_{12} and C_{14} acids. When this factor is applied for calculation of the extinction coefficient for the highly unsaturated acids, there results λ_{max}, mμ ($\epsilon \times 10^{-3}$), 300 (37.6), 314 (47.0), 329 (43.5), 346 (39.4).

The highly unsaturated components, which had not appeared in gas chromatography on account of their instability, were revealed by hydrogenating a freshly prepared sample of esters and gas chromatographing the resultant saturated esters. Such ester samples contained 10–15% each of methyl dodecanoate and methyl tetradecanoate.[4] The ultraviolet spectrum of the esters of the aje acids (Figure 1) shows four strong maxima (300, 314, 329, and 346 mμ) and two weaker absorptions (272, 285 mμ). This spectrum is more complex and gives a band at longer wavelength than reported for α-parinaric acid,[5] which has four conjugated double bonds in an unsubstituted chain. There appears to be no report of an unbranched, acyclic conjugated pentaunsaturated system in natural occurrence; however, a pentaunsaturated acid has been isomerized with alkali to generate the conjugated system.[6] Ultraviolet absorption after the alkali isomerization showed six absorption bands in essentially the same positions shown in Figure 1 (270, 285, 300, 313, 330, and 345 mμ); furthermore, the stronger bands were those four at longer wavelengths. With strong alkali isomerization, the strongest absorption was at 330 mμ, but in weak alkali the strongest absorption was at 313 mμ, as is the case in Figure 1. Thus, the acids from aje must have a conjugated system of five double bonds. No reliable conclusions may be reached concerning the geometry of this unsaturated system.

As one attack on location of the conjugated system revealed by the ultraviolet absorption, a freshly prepared sample of esters was catalytically reduced with deuterium, and mass spectra were determined for the esters of the saturated C_{12} and C_{14} acids, which had been separated by gas chromatography. Since catalytic deuteration gives extensive indiscriminate exchange of deuterium for hydrogen, this procedure is quite inferior to specific reduction of the alkene linkage with deuteriohydrazine;[7] however, in the present instance, hydrazine reduction was noncompetitive with polymerization. In spite of the scrambling of deuterium which occurs on catalytic deuteration, the mass spectrum (Table I) of the ester of the C_{14} acid proved definitive for location of the carbon on which the conjugated system begins.

(4) In one specimen of aje (*cf.* Experimental Section), the original esters contained a trace of dodecanoate and about 15% of tetradecanoate; however, in this sample presence of the highly unsaturated esters was revealed by an increase of dodecanoate and tetradecanoate after hydrogenation.

(5) M. O. Bagby, C. R. Smith, Jr., and I. A. Wolff, *Lipids*, **1**, 263 (1966).

(6) S. F. Herb and R. W. Riemenschneider, *J. Amer. Oil Chem. Soc.*, **29**, 456 (1952).

(7) N. Ding-Nguyên, R. Ryhage, and S. Ställberg-Stenhagen, *Ark. Kemi*, **15**, 433 (1960).

TABLE I

PARTIAL MASS SPECTRUM OF CATALYTICALLY DEUTERATED METHYL TETRADECAPENTAENOATE

m/e	%[a]	*m/e*	%[a]
74[b]	100	131	3.0
75	44	132	2.0
76	17	143[c]	1.0
87[c]	46	144	2.5
88	44	145	4.2
89	19	146	4.8
101[c]	7.5	147	4.0
102	6	157[c]	<0.2
103	4	158	<0.2
115[c]	1.6	159	1.0
116	2.5	160	1.5
117	1.7	161	1.8
129[c]	1.0	162	1.0
130	2.1		

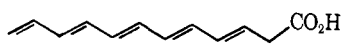
[a] Relative abundance in relation to the most abundant ion. [b] The rearrangement ion, $CH_2{=}C(OH)OCH_3$, with no deuterium. [c] Ester fragments, $(CH_2)_nCO_2CH_3$, with no deuterium.

From the data in Table I, it is seen that the rearrangement ion, whose *m/e* is 74 in absence of deuterium, is quite enlightening. Since this fragment containing no deuterium is in much the greatest abundance, and since two of its hydrogen atoms are from the α position and one is from the γ position, this establishes absence of double bonding to the α, β, and γ carbons. This is supported by examination of the simple ester fragments, for the fragments containing respectively two and three methylene groups give the undeuterated ion in greatest abundance. In contrast, the ester fragments with four, five, six, and seven methylene groups give most abundant ions respectively with one, two, three, and four deuterium atoms. Thus, the double bond system must start at carbon-5. Surprisingly, the most abundant molecular ion was of *m/e* 250, two less than required for ten deuterium atoms.

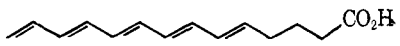
The mass spectrum of the deuterated C_{12} acid is less definitive than that of the C_{14} acid; however, it remains possible to assign the doubly bonded carbon nearest to the carbonyl. The rearrangement ion is the most informative feature of the mass spectrum of this ester. For *m/e* of 74, 75, and 76 relative abundances of the respective ions were 100, 77, and 19%. It will be noted that the relative abundance of 75 is nearly twice that in Table I but less than the abundance of 74. This indicates that deuterium from the reduction of a double bond is not at the α position, for this would make the ion at 75 more abundant than that at 74 (much more, with no scrambling). If the deuterium is rearranged from the γ position, however, there would also be one hydrogen at this position, and the hydrogen would be more prone to rearrange on account of its lower bond energy. Furthermore, relative abundances of ions at *m/e* 87, 88, and 89 were respectively 36, 57, and 46%. These data indicate a double bond at the β position (compare with Table I); therefore, the conjugated system starts at the β,γ position.

According to the ultraviolet spectrum and mass spectrometry of the deuterated esters, the highly un-

saturated acids, which we propose to call C_{12}-ajenoic acid and C_{14}-ajenoic acid, have the structures shown in **1** and **2**. In order to obtain classical degradative



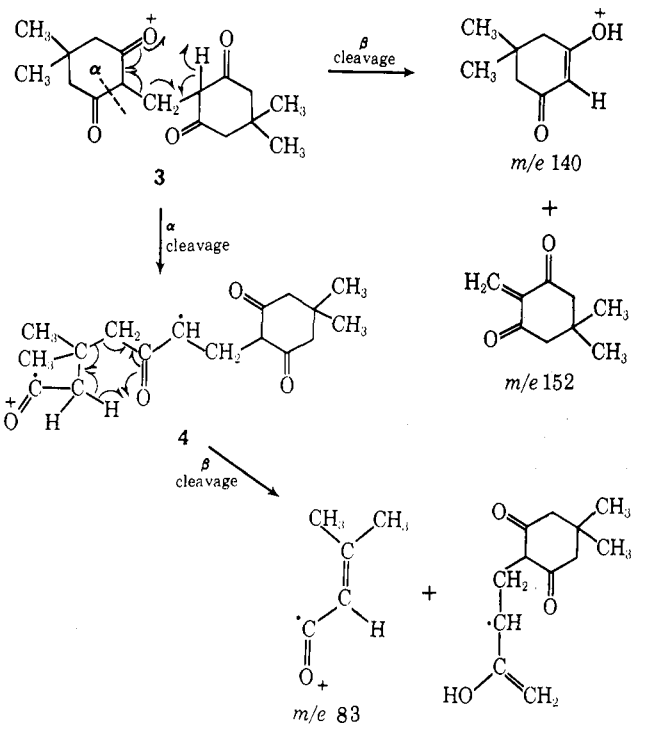
1



2

evidence in support of these structures, the ajenoic acids were separated from the bulk of the higher molecular weight acids by low temperature precipitation of the latter, and the ajenoic acids were subjected to ozonization. In one set of experiments, the ozonide was worked up for formaldehyde which was isolated as the dimedone adduct in good yield. Comparison with an authentic sample of this adduct was by melting point, as well as mass spectrum.

The mass spectrum of the formaldehyde–dimedone adduct is of interest in that the most abundant ion, *m/e* 83, as well as the other two prominent ions in the spectrum, *m/e* 140 (35%) and *m/e* 152 (55%), can be formulated on the basis of classical α and β cleavages, in spite of the complexity of the molecule. In structure **3**, the β cleavage, involving hydrogen



3 **4**

rearrangement as depicted by the arrows, separates the rings and yields the fragments of the observed masses. A somewhat different shift of electrons leaves the charge on the *m/e* 152 fragment, and this effect is often observed in instances where the alkene fragment contains additional oxygen or nitrogen atoms. Both an α and a β cleavage are required to yield the base peak of *m/e* 83. If an α cleavage of structure **3** occurs at the position indicated by the dotted line, the resultant structure **4**, in the conformation depicted, may yield the *m/e* 83 ion *via* the β cleavage indicated by the arrows. The stability of the *m/e* 83 ion may be ascribed to the possibility of delocalization of electrons to yield a tertiary carbonium ion (charge on carbon β to carbonyl).

As further confirmation of structure, in another procedure the initial ozonization products from the aje acids were oxidized by permanganate according to a previously developed procedure,[8] and the resultant acids esterified. This yielded dimethyl malonate (from structure **1**) and dimethyl glutarate (from structure **2**). The diesters were collected from gas chromatography and their mass spectra compared with authentic samples.

In view of the unusual structures encountered in the ajenoic acids, the esters of the unsaturated C_{18} acids were also subjected to ozonization, permanganate oxidation, and esterification. Identification by gas chromatography and mass spectrometry established the cleavage products as methyl hexanoate, methyl nonanoate, and dimethyl azelate. Thus, the unsaturated C_{18} acids prove to be the ubiquitous oleic and linoleic acids.

Since there became available to us a sample of the fat from Guatemala,[9] and since there is some opinion that this coccid is different from that in Mexico, we determined the type and distribution of acids in this sample of aje or nije. As described in the Experimental Section, the composition of the Guatemalan fat was surprisingly similar to that from Mexico. Of course this observation raises very serious doubts that the coccids are different.

Experimental Section[10]

Aje Specimens.—The aje fat is extracted from mature female coccids by cooking and crushing them in water. The mass of fat is separated from solid matter by straining, washed with water, formed into cakes, and wrapped in leaves or cornhusks for marketing. The "refined" aje, used as a medicinal unguent, is a lighter yellow color than the yellow-brown material used for gourd painting. The "refining" process probably consists of further water washing and straining of the melted fat for removal of insoluble foreign matter.

Two specimens were employed in most of these investigations. One was a refined sample obtained by Mrs. Jenkins[1] in 1963 at Mitla in Oaxaca but was brought there from Choapan, in the Sierra Juarez, by an itinerant trader. The other sample was unrefined aje with a heavy crust on the outside. This sample was bought by Mrs. Jenkins in Chiapas in 1962. The major difference in the fatty acid content of the two samples was the occurrence of about 15% of tetradecanoic acid and a lower ratio of eicosanoic acid in the unrefined sample. The sample of ni-in, originating in Rabinal, Guatemala, was obtained by Mrs. Jenkins in Oct 1968.

Samples removed from the soft center of the aje cakes were used. These contained 10–20% of ether-insoluble material (presumably polymer). No free fatty acids were removed by countercurrent extraction of the ether solution with dilute aqueous potassium hydroxide, using a sequence of three Kies tubes.[11]

Acids from Saponification of Aje.—In a typical procedure 1.1 g of aje fat was saponified by heating under reflux in a nitrogen atmosphere for 2 hr with 10 ml of 2 *M* methanolic KOH. Work-

(8) J. Cason and W. T. Miller, *J. Biol. Chem.*, **238**, 883 (1963).

(9) The Mayan word for this fat is ni-in, and the fat is known as nije in the Baja Verapaz region of Guatemala from which Mrs. Jenkins obtained this sample. In Guatemala, the coccid is known as *Llaveia bouvari*.

(10) Mass spectra were determined by Miss Sherri Firth on a CEC Model 21-103c instrument, with the inlet heated to about 180° and the ionizing voltage at 70 eV. The instrument had been equipped with narrower slits and an ion multiplier and otherwise modified to give unit resolution to about 600. Gas chromatography was on an Aerograph Model A-90P. Ozonizations were performed at −70°, using ozone from a Welsbach laboratory ozonator, Model T-23.

(11) M. W. Kies and P. L. Davis, *J. Biol. Chem.*, **189**, 637 (1951).

up for acids yielded 0.8 g of yellow solid. Neutral components were not examined. Classical acid-catalyzed esterification gave poor yields of ester, with much polymer; so base-catalyzed esterification[3] was preferred. For this purpose, 1.0 g of crude acids was heated on a steam bath for 5 min with 10 ml of methanol, 2.6 g of tris-(2-hydroxypropyl)amine, and 1.6 g of dimethyl sulfate. Work-up for neutral material yielded 0.84 g of crude esters which were too viscous for injection in glpc; so they were injected in about 10% solution in benzene.

On glpc of the esters with a 0.25 in. × 5 ft column, 20% SE-30, 225°, helium flow rate 60 cc/min, bands were observed at retention times of 22.8, 25.2, 46.2 min. A semilog plot of retention times for methyl esters of C_{12} to C_{18} acids showed coincidence of the final two bands with stearate and eicosanoate, while the first band had the retention time of unsaturated esters of the C_{18} acid. With use of a $^3/_8$ in. × 5 ft column, 20% DEGS, 200°, helium flow rate 110 cc/min, bands were observed at retention times of 24.0, 27, 32.9, 40.7 min. In comparison with known compounds, the first and last bands corresponded with stearate and eicosanoate, while the second and third corresponded with oleate and linoleate.

A 20-mg sample of the second and third peaks on DEGS was collected and hydrogenated in glacial acetic acid solution, with 10% Pd-on-charcoal catalyst, at an initial pressure of 30 psi. Gas chromatography of the hydrogenated ester have a single peak of the retention time of stearate; therefore, the 27- and 32.9-min bands on DEGS are ascribed to **monounsaturated** and **nonconjugated diunsaturated octadecanoates**. Position of unsaturation was determined by ozonization, as described below.

A sample of material collected from the 25.2-min band on silicone had mp 37–38.5°, no depression on admixture with an authentic sample of **methyl stearate**. Ir spectra of the authentic and isolated samples were also identical.

A sample (0.6 mg) of material collected from the 46.2-min band on silicone was submitted for mass spectrum.[10] The base peak (most abundant ion) was of m/e 74, the rearrangement ion from a methyl ester; the molecular ion was of m/e 226 (10% of base peak), the molecular weight of **methyl eicosanoate**. The ion of m/e 87, $CH_2CH_2CO_2CH_3$, was of 54% relative abundance. For the fragment, $(CH_2)_nCO_2CH_3$, every ion was relatively prominent from $n = 2$ to $n = 17$, with the two highest mass ones (283, 2.75%; 297, 1%) more conspicuous than their lower neighbors, since 283 represents M − 43 (α, β, and γ carbons + H) and 297 represents M − 29 (α and β carbons + H). Also present was 295 (1.6%), which is M − 31 (OCH_3). These are all the features characteristic of the mass spectrum of the methyl ester of a normal carboxylic acid, and they were accompanied by no features indicating a branch in the chain.

By combining glpc data on aje methyl esters with that on the hydrogenated methyl esters, composition of the fat was determined. In Table II are the average values for composition,

TABLE II
COMPOSITION OF AJE FAT

Acid	% of total[a] Mexican	Guatemalan
Dodecanoic	Trace	Nil
C_{12}-Ajenoic	13	11
Tetradecanoic	7.5	7
C_{14}-Ajenoic	10	9.5
Octadecanoic	39	
Oleic	11	67.5
Linoleic	14	
Eicosanoic	5.5	5

[a] Composition is based on relative areas under the peaks in glpc tracings, with no correction for any variation of response with molecular weight.

based on several lots of the refined sample and two lots of the unrefined sample from Mexico. One lot of the Guatemalan sample was examined.

Perhydroajenoic Esters.—In a typical hydrogenation (or deuteration), 2.6 g of freshly prepared aje methyl esters was reduced at an inital pressure of 30 psi, in solution in 44 ml of glacial acetic acid, with 0.2 g of 10% Pd-on-charcoal catalyst. After hydrogenation had been continued for 24 hr, the pressure drop amounted to 1.3 mol of hydrogen per mole of esters (based on average mol wt 300). After work-up, including filtration of polymer from ether solution, 2.1 g of semisolid esters were obtained. For collection in glpc of the reduced ajenoic esters, there was used a 0.25 in. × 5 ft column, 20% SE-30, 210°, helium flow rate 60 cc/min; retention times of 4.8 and 9.2 min, identical with those of authentic samples of methyl laurate and methyl myristate.

The ir spectrum of each sample was characteristic of a methyl alkanoate, nearly identical with the spectrum of methyl stearate. The nmr spectrum of the perhydroajenoates (12 μl in 0.3 ml of CCl_4, TMS internal standard, Varian A-60 instrument) showed the characteristic spectrum of a normal long-chain methyl ester, with the two characteristic features: the triplet centered at τ 9.12 (terminal methyl hydrogens), and the triplet centered at τ 7.80 (α hydrogens).

Pertinent features of the mass spectrum of the deuterated methyl C_{14}-ajenoate are incorporated in Table I; a similar spectrum was obtained for the C_{12}-ajenoate.

Ozonization of Ajenoic Acids.—For fractionation of the aje acids, following a published procedure,[12] 1.75 g of freshy prepared acids in about 25 ml of acetone was cooled to 0°. After standing for a period, the saturated acids which crystallized were removed by suction filtration. After the filtrate had been cooled to −50°, the precipitated fraction consisting largely of the unsaturated C_{18} acids was removed, and then the ajenoic acids were recovered by evaporation of the final filtrate.

A 100-mg sample of the ajenoic acid fraction, dissolved in 50 ml of dichloromethane, was treated with ozone during 30 min until excess ozone had been applied. The ozonide was reduced by addition of 5 ml of triethyl phosphite. After a solution of 160 mg of dimedone in 2 ml of ethanol and 17 ml of water had been added, the reaction mixture was allowed to warm to room temperature, then heated on a steam bath for 15 min, and finally left at room temperature for 2 hr. The solid which had formed was collected by suction filtration, washed with water, and recrystallized from ethanol: yield 28 mg; mp 189–190°, no depression on admixture with an authentic sample of the same melting point. The mass spectra of the isolated and authentic samples were essentially identical, except for a less abundant molecular ion (m/e 292) in the isolated sample.

For isolation of esters of dibasic acids, a 350-mg sample of the ajenoic acid fraction was ozonized as described above, but the dichloromethane solution of the ozonide was decomposed by heating under reflux for 1 hr with 20 ml of water. The products recovered by evaporation of the dichloromethane solution were stirred overnight at room temperature with 500 mg of potassium permanganate and 200 ml of acetone. Excess permanganate was decomposed by addition of 5 ml of methanol and stirring for an additional 1 hr. The reaction mixture was worked up by acidification to congo red, addition of sufficient sodium metabisulfite to decompose manganese dioxide, and extraction with ether. Evaporation of the dried ether solution yielded 300 mg of viscous oil which was esterified by heating under reflux for 2 hr with 100 ml of methanol containing 5 ml of concentrated H_2SO_4. Esters recovered by a normal work-up were chromatographed on a 0.25 in. × 5 ft column, 20% DEGS, 120°, helium flow rate 100 cc/min. Retention times of 1.6 min for dimethyl malonate and 3.7 min for dimethyl glutarate were the same as those observed for authentic samples of these esters. Samples collected from gas chromatography were used for mass spectra; results were identical with those from authentic samples. In each ester, the most abundant ion was M − OCH_3 (m/e 101 and 129); other prominent ions were those expected except for glutarate, which gave a more abundant M + 1 ion (m/e 161) than M, even at low vapor density.

Ozonization of Esters of the Unsaturated C_{18} Acids.—Since the C_{18} acids were too insoluble in dichloromethane at −70° for satisfactory ozonolysis, a 400-mg sample of this fraction was base esterified as described for the mixed aje acids. The resultant 310 mg of esters was ozonized, worked up, and oxidized with permanganate as described for the ajenoic acids. The methyl esters of the resultant acids were chromatographed on a 0.25 in. × 5 ft column, 20% DEGS, 130°, helium flow rate 100 cc/min. Retention times: methyl hexanoate, 2 min; methyl nonanoate, 6.9 min; dimethyl azelate, 21.5 min. Mass spectra of the collected samples and authentic samples were identical, and the ions

(12) C. Y. Hopkins and M. J. Chisholm, *J. Amer. Oil Chem. Soc.*, **45**, 176 (1968).

were those expected, with the rearrangement ion (*m/e* 74) the most abundant. Again the diester gave M + 1 (*m/e* 217) considerably more abundant than M.

Registry No.—**1**, 30409-26-8; **2**, 30345-96-1; deuterated methyl tetradecapentaenoate, 30345-97-2.

Acknowledgment.—We wish to express our appreciation to Dr. C. R. Smith, Jr., Northern Regional Research Laboratory, Peoria, Ill., who, when the manuscript was originally submitted, included in his referee report recommendations which led to our establishing the correct structures for the ajenoic acids.

Structural Modifications of Isosteviol. Partial Synthesis of Atiserene and Isoatiserene[1]

ROBERT M. COATES* AND EDWARD F. BERTRAM

Department of Chemistry, University of Illinois, Urbana, Illinois 61801

Received December 3, 1970

By means of functional interconversions in ring D of the tetracyclic diterpene isosteviol (*ent*-16-oxobeyeran-19-oic acid, **1**), various 15- and 16-substituted methyl *ent*-beyeran-19-oates (**3**–**7**) have been prepared. Ring C functionalization at positions 12 and 14 has been accomplished by degradation of isosteviol to the unsaturated tricyclic tosylate esters **14c** and **15c** [methyl *ent*-8α-(2′-tosyloxyethyl)-13-methyl-12-podocarpen-19-oate and its Δ^{13} double bond isomer] followed by recyclization. Buffered formolysis of **14c** at room temperature affords after partial hydrolysis methyl *ent*-16β-hydroxyatisan-19-oate (**16a**), which at 85° in formic acid rearranges to methyl *ent*-12β-formyloxybeyeran-19-oate (**18d**). Formolysis of **15c** at 80° gives a tetracyclic formate formulated as methyl *ent*-14β-formyloxybeyeran-19-oate (**17d**). Dehydration of **16a** produces the exocyclic and endocyclic unsaturated esters **21** and **22** which were separately converted into atiserene (**25**) and isoatiserene (**28**).

In order to examine the biogenetic-like rearrangements within the kaurene and atiserene family of tetracyclic diterpenes,[2] we needed synthetic access to both 12β- and 16β-beyerane (hibaane) derivatives with functional groups suitable for the generation of carbonium ion intermediates. This paper describes the conversion of the relatively available diterpene, isosteviol (**1**, *ent*-16-oxobeyeran-19-oic acid),[3–6] into the 12β-hydroxy (**18a**), 16β-hydroxy (**7a**), and 16-amino (**4**) esters. The synthetic route to the 12β-hydroxy ester, proceeding by way of methyl *ent*-16β-hydroxyatisan-19-oate (**16**), opened the way to a partial synthesis of atiserene (**25**) and isoatiserene (**28**).[2b,7]

Since sodium borohydride reduction of the 16-carbonyl group of isosteviol methyl ester **2** affords exclusively the undesired endo (α) hydroxy ester **5a**,[8] the investigation of other approaches was necessary. Hydroboration of the unsaturated ester **8** with disiamylborane[9] in tetrahydrofuran gave rise to a 2:3 mixture of the two exo (β) hydroxy esters **6a** and **7a**. A 3:2 distribution of the 15β and 16β isomers has been reported for hydroboration of hibaene (*ent*-15-beyerene, **8** with CH_3 in place of CO_2CH_3) with diborane.[10] Although a partial separation of the mixture could be achieved by column chromatography of the corresponding acetates **6b** and **7b**, the purification was both tedious and inefficient. Reduction of isosteviol methyl ester by the Meerwein–Ponndorf–Verley method under equilibrating conditions[11] for prolonged periods afforded a mixture (about 1:1) of **5a** and **7a**, which again was partially separated by repeated chromatography of the acetate derivatives **5b** and **7b**. However, by recycling the undesired endo isomer along with mixed factions, a satisfactory yield (59%) of **7b** was realized. Amino ester **4** was prepared by reduction of the oxime **3** of isosteviol methyl ester with sodium in isopropyl alcohol (Scheme I).

The stereochemistry of the hydroxyl group in **5**, **6**, and **7** is assigned on the assumption that attacking reagents in irreversible reactions will approach the 15 or 16 positions of the beyerane nucleus from the exo (β) direction. Although there exists extensive precedent for high stereoselectivity in a wide variety of reactions involving several D ring functional groups in tetracyclic diterpenoids,[12] independent stereochemical evidence is, to our knowledge, limited to recent nmr data (car-

(1) Taken in part from the Ph.D. thesis of E. F. B., University of Illinois, 1970.

(2) (a) R. M. Coates and E. F. Bertram, *Tetrahedron Lett.*, 5145 (1968); (b) R. M. Coates and E. F. Bertram, *Chem. Commun.*, 797 (1969); (c) manuscript in preparation.

(3) Isolation: (a) H. B. Wood, Jr., R. Allerton, H. W. Diehl, and H. G. Fletcher, Jr., *J. Org. Chem.*, **20**, 875 (1955); (b) M. Ruddat, E. Heftmann, and A. Lang, *Arch. Biochem. Biophys.*, **110**, 496 (1965).

(4) Source of *Stevia Rabaudiana* Bertoni (dried leaves and stems or extract): Mr. Luis Enrique de Gesperi, Empresas Ago-Industrales, Asuncion, Peru. We are grateful to Mr. de Gesperi for a sample of the extract.

(5) Structure: (a) E. Mosettig, U. Beglinger, F. Dolder, H. Lichti, P. Quitt, and J. A. Waters, *J. Amer. Chem. Soc.*, **85**, 2305 (1963); (b) J. R. Hansen, "The Tetracyclic Diterpenes," Pergamon Press, Oxford, England, 1968, pp 23–25.

(6) The numbering system used throughout this paper conforms to the recommendations ("The Common and Systematic Nomenclature of Cyclic Diterpenes," 3rd revision, Oct 1968; Adenda and Corrigenda, Feb 1969) prepared by J. W. Rowe (Forest Products Laboratory, Forest Service, U. S. Department of Agriculture, Madison, Wisc. 53705). Both common and systematic names are used in the text as appropriate; complete systematic names appear in the Experimental Section. We are grateful to Dr. Rowe for copies of these recommendations.

(7) A. A. Kapadi, R. R. Sobti, and S. Dev, *Tetrahedron Lett.*, 2729 (1965); A. A. Kapadi and S. Dev, *ibid.*, 2751 (1964).

(8) J. R. Hanson, *Tetrahedron*, **23**, 793 (1967).

(9) (a) G. Zweifel, K. Nagase, and H. C. Brown, *J. Amer. Chem. Soc.*, **84**, 190 (1962); (b) H. C. Brown and G. Zweifel, *ibid.*, **83**, 1241 (1961).

(10) R. R. Sobti and S. Dev, *Tetrahedron Lett.*, 3939 (1966).

(11) C. F. Wilcox, Jr., M. Sexton, and M. F. Wilcox, *J. Org. Chem.*, **28**, 1079 (1963).

(12) The following selection of references include osmium tetroxide oxidation, epoxidation, hydride reduction, catalytic hydrogenation, sensitized oxygenation, hydroboration, and Grignard addition: (a) P. K. Grant and R. Hodges, *Tetrahedron*, **8**, 261 (1960); (b) J. R. Hanson, *J. Chem. Soc.*, 5061 (1963); (c) L. H. Briggs, B. F. Cain, R. C. Cambie, B. R. Davis, P. S. Rutledge, and J. K. WilmsHurst, *ibid.*, 1345 (1963); (d) R. A. Finnegan, *J. Org. Chem.*, **26**, 3057 (1961); (e) L. H. Briggs, R. C. Cambie, and P. S. Rutledge, *J. Chem. Soc.*, 5374 (1963); (f) L. H. Briggs, B. F. Cain, R. C. Cambie, and B. R. Davis, *ibid.*, 1840 (1962); (g) H. Vorbreugen and C. Djerassi, *J. Amer. Chem. Soc.*, **84**, 2990 (1962); (h) B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 2944 (1963); (i) M. Barnes and J. MacMillan, *ibid.*, *C*, 361 (1967); (j) J. R. Hanson, *Tetrahedron* **23**, 801 (1967); (k) K. Mori and M. Matsui, *ibid.*, **24**, 3095 (1968); (l) R. A. Appleton, P. A. Gunn, and R. McCrindle, *J. Chem. Soc.*, 1148 (1970), and ref 5 8, and 10.