Laballenic Acid. A New Allenic Acid from *Leonotis nepetaefolia* Seed Oil[1]

M. O. BAGBY, C. R. SMITH, JR., AND I. A. WOLFF

Northern Regional Research Laboratory,[2] Peoria, Illinois 61604

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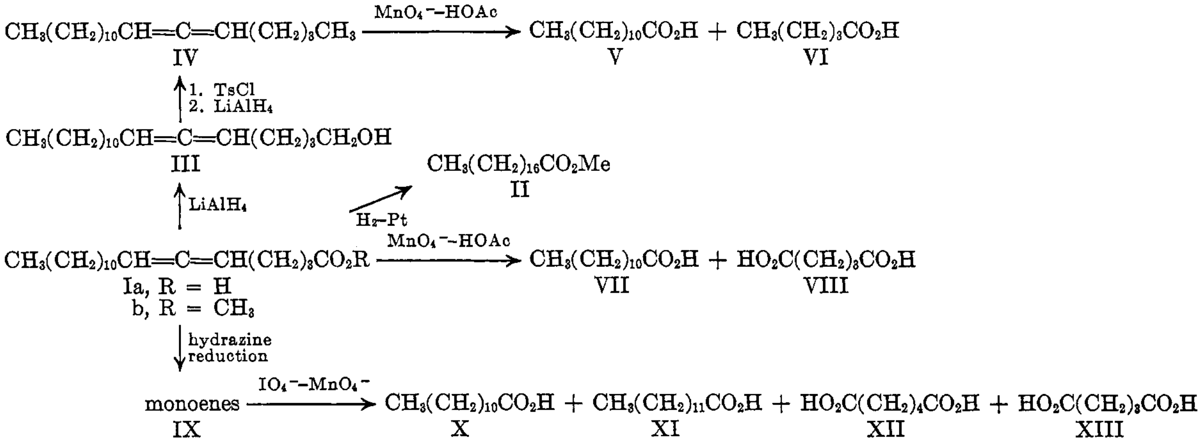
Seed oil from *Leonotis nepetaefolia* contains 16% of laballenic [(−)-5,6-octadecadienoic] acid. This is the first example of a natural C_{18} allene from higher plants. An anomalous result with periodate–permanganate oxidation was found in which glutaric acid, an expected cleavage product, was not detected. Laballenic acid was very stable to alkali.

In a preliminary communication,[3] we reported isolation of an allenic acid from seed oil of *Leonotis nepetaefolia* L. (R. Br.) (family *Labiatae*). It was suggested that this acid, laballenic acid (Ia), might be 2,3-methylene-4,5-heptadecadienoic acid. This paper describes additional work which shows that laballenic acid is (−)-5,6-octadecadienoic acid (see Scheme I).

SCHEME I



$$\begin{tikzcd}
\underset{\text{IV}}{CH_3(CH_2)_{10}CH{=}C{=}CH(CH_2)_3CH_3} \arrow[r, "MnO_4^-{-}HOAc"] & \underset{\text{V}}{CH_3(CH_2)_{10}CO_2H} + \underset{\text{VI}}{CH_3(CH_2)_3CO_2H} \\
\underset{\text{III}}{CH_3(CH_2)_{10}CH{=}C{=}CH(CH_2)_3CH_2OH} \arrow[u, "\text{1. TsCl; 2. }LiAlH_4"] & \underset{\text{II}}{CH_3(CH_2)_{16}CO_2Me} \\
\underset{\text{Ia, }R = H;\ \text{b, }R = CH_3}{CH_3(CH_2)_{10}CH{=}C{=}CH(CH_2)_3CO_2R} \arrow[u, "LiAlH_4"] \arrow[ur, "H_2\text{-}Pt"'] \arrow[r, "MnO_4^-{-}HOAc"] \arrow[d, "\text{hydrazine reduction}"] & \underset{\text{VII}}{CH_3(CH_2)_{10}CO_2H} + \underset{\text{VIII}}{HO_2C(CH_2)_3CO_2H} \\
\underset{\text{IX}}{\text{monoenes}} \arrow[r, "IO_4^-{-}MnO_4^-"] & \underset{\text{X}}{CH_3(CH_2)_{10}CO_2H} + \underset{\text{XI}}{CH_3(CH_2)_{11}CO_2H} + \underset{\text{XII}}{HO_2C(CH_2)_4CO_2H} + \underset{\text{XIII}}{HO_2C(CH_2)_3CO_2H}
\end{tikzcd}$$

Mixed methyl esters, derived from the oil by acid-catalyzed transesterification, were fractionated by countercurrent distribution (c.c.d.) with a solvent system of hexane–acetonitrile. In the presence of platinum catalyst, methyl laballenate (Ib, *ca.* 95+% pure) readily absorbed 2 mole equiv. of hydrogen to yield stearate (II). The infrared spectrum of methyl laballenate (Ib) showed allene[4] absorption at 5.12 μ. A single maximum between 175 and 300 mμ occurred at 181.5 mμ, thus indicating absence of conjugated unsaturation. The presence of strong optical activity, $[\alpha]_D$ −47.3°, supported the spectral evidence that I was an allene.

Partial reduction of Ia with hydrazine[5–9] and subsequent fractionation of the products by c.c.d. yielded saturated acid and a mixture of two monoenes (IX) containing 19.9% of isolated *trans* unsaturation.[10] Periodate–permanganate oxidation[11] of IX yielded lauric, tridecanoic, glutaric, and adipic acids as cleavage products. Thus, the presence of the allene grouping was confirmed, and the position of the unsaturation in the parent molecule was indicated.

Repeated attempts to cleave Ib or Ia with periodate–permanganate yielded the expected lauric acid together with small amounts of several minor components but none of the anticipated glutaric acid. Cleavage of methyl laballenate with permanganate in acetic acid[12] did yield the expected products—lauric and glutaric acids. The anomalous result obtained from periodate–permanganate oxidation of laballenic acid was unexpected; however, a similar anomaly has been experienced with β-hydroxyadipic acid.[13]

Efforts to confirm further the structure of laballenic acid were directed toward reduction of the carboxyl group as outlined by Hands and Bartley.[14] The allenic hydrocarbon product IV was oxidatively cleaved with permanganate in acetic acid to yield primarily lauric and valeric acids. These results indicate that the structure of laballenic is (−)-5,6-octadecadienoic acid.

In our previous communication,[3] we reported that the saturated ester had an infrared spectrum which differed from that of methyl stearate. This difference is believed to be due to a contaminant, since subsequent preparations were identical with methyl stearate.

During this investigation it seemed relevant to isomerize laballenic acid with alkali. Conditions used by Crowley[15] to isomerize 3,4-hexadienoic and 3,4-pentadienoic acids to sorbic and 2,4-pentadienoic acids, respectively, caused no appreciable modification of laballenic acid even after 4 hr. at reflux temperature with

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[2] A laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

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10% sodium hydroxide. Limited isomerization was effected with 10% potassium hydroxide in ethylene glycol at about 170°. Potassium *t*-butoxide in dimethyl sulfoxide, known to facilitate ionic reactions,[16] did cause isomerization to take place. However, rearrangement of the allene grouping was not complete even after 0.5 hr. at about 85–90°. G.l.p.c. analysis and spectral evidence indicated products containing these structures: $-C{\equiv}C-$, $-CH{=}CH-CH{=}CH(CH_2)_nCO_2R$, $-CH{=}CH-CH{=}CHCO_2R$, and $-CH{=}CH(CH_2)_nCH{=}CHCO_2R$. When the reaction was carried out at room temperature, the preponderant product was acetylenic acids.

Most naturally occurring allenic compounds previously known are found as fungal metabolites,[17–20] in which the allene grouping is part of a conjugated system. 8-Hydroxy-5,6-octadienoic acid has been reported to be a constituent of *Sapium sebiferum* seed oil[21]; however, laballenic acid is the first known C_{18} acid with an allene group. Together, the acid from *S. sebiferum* and laballenic acid are the only known nonconjugated allenes found in nature. Hagemann, *et al.*,[22] have presented evidence that laballenic acid probably has general occurrence in the subfamily *Stachydoideae* of the *Labiatae*.

Experimental Section

Infrared spectra were determined with Infracord Model 137[23] or Model 337 spectrophotometers, n.m.r. spectra were determined with a Varian A-60 spectrometer on carbon tetrachloride solutions containing tetramethylsilane, and ultraviolet spectra were determined on isooctane solutions with a Beckman DK-2A far-ultraviolet spectrophotometer. Melting points were determined with a Fisher-Johns block and are uncorrected. G.l.p.c. analyses were carried out as described by Miwa, *et al.*[24] Free acids were identified by comparing retention times of observed peaks with those found for components of a standard mixture or, in some instances, by injecting admixtures of unknown and authentic specimens. Quantities are reported as area per cents.

Preparation of Mixed Methyl Esters.—Coarsely ground seed of *Leonotis nepetaefolia* was extracted overnight in a Soxhlet apparatus with pentane–hexane (b.p. 33–57°). The bulk of the solvent was removed under nitrogen on a steam bath, and the remainder *in vacuo* with a rotating evaporator.

L. nepetaefolia oil (40.43 g.) was transesterified by refluxing 2 hr. under nitrogen with methanol containing 1% of sulfuric acid. The methanolic solution was diluted with water then extracted with ethyl ether. The ether extract was dried over sodium sulfate, and then the solvent was removed *in vacuo* to yield 37.15 g. of esters. Additional quantities of esters were prepared similarly as needed. An alternative procedure consisted of saponification by refluxing 0.5 hr. under nitrogen with 2 *N* ethanolic potassium hydroxide. Unsaponifiable material (2.4%) was removed, and free fatty acids were obtained in the usual manner. Methyl esters were subsequently prepared by acid catalysis.

Isolation of Methyl Laballenate.—Mixed methyl esters (37.15 g.) were dissolved in mutually saturated hexane (150 ml.) and acetonitrile (600 ml.) and subjected to a 450-transfer c.c.d. in a 200-tube automatic Craig-Post apparatus. Esters were divided evenly among the first 15 tubes, and 40 ml. (full in the decant position) of lower phase was placed in each of the remaining tubes. Automatic operation of the instrument introduced 10 ml. of equilibrated hexane (upper phase) to tube zero at each transfer stage. As the upper phase progressed past tube 200, it was decanted into an automatic fraction collector; two transfers per tube were combined and successively collected until 130 fractions had been obtained. Solvent was evaporated, under reduced pressure, from the contents of selected tubes. G.l.p.c. analyses of significant fractions indicated that most of the methyl laballenate was present in transfers 300 through 360. These fractions were combined and subjected to a second c.c.d. with a combination of recycle–single withdrawal techniques.[25] After 550 transfers, fractions were collected as above until 850 transfers had been made. Transfers 692 through 723 were combined to give 1.79 g. of a fraction containing 96.8% of methyl laballenate, 1.4% of methyl oleate, 1.3% of a C_{16} monoene, and 0.5% of methyl myristate: $[\alpha]^{26}\text{D}$ $-47.3°$ (*c* 1.8, ethanol); $\lambda_{\max}$ 181.5 mμ (ϵ 19,930) and 5.12 μ (allene).[4] Additional methyl laballenate (95.1–96.8% purity) was prepared in a similar manner. *p*-Bromophenacyl laballenate, m.p. 47.0–47.5°, from aqueous ethanol, was prepared by the usual technique.[26]

Anal. Calcd. for $C_{26}H_{37}BrO_3$. C, 65.40; H, 7.81; Br, 16.74. Found: C, 65.64; H, 7.80; Br, 16.93.

Methyl laballenate (0.397 g.) in ethanol readily absorbed 2.0 mole equiv. of hydrogen in the presence of platinum catalyst to yield methyl stearate (0.385 g.), m.p. 35.5–36.5°. Its infrared spectrum, n.m.r. spectrum, and g.l.p.c. characteristics were identical with those of authentic methyl stearate.

Anal. Calcd. for $C_{19}H_{38}O_2$: C, 76.45; H, 12.83. Found: C, 76.40; H, 12.62.

Periodate–Permanganate Oxidation of Ib.[11]—Methyl laballenate (0.047 g.) was stirred 16 hr. with 25 ml. of *t*-butyl alcohol, 25 ml. of stock oxidant, and 0.044 g. of potassium carbonate in 10 ml. of water. The oxidation was terminated by chilling, acidifying, and then reducing the excess oxidants with sodium bisulfite. Potassium hydroxide (2 g.) was added and the alcohol was removed *in vacuo*. The aqueous solution was acidified, saturated with sodium chloride, and then extracted with ethyl ether (six 30-ml. portions). The combined ether extract was dried over sodium sulfate. G.l.p.c. analyses of methyl esters, prepared by reaction with diazomethane,[27] showed methyl laurate as the only major cleavage product, 90+%. Similarly, oxidation of a less pure sample (63% of methyl oleate and 35% of methyl laballenate) yielded pelargonic, azelaic, and lauric acids in a ratio of about 2:2:1.

Permanganate in Acetic Acid Oxidation of Ib.[12]—To a stirred solution of 1.043 g. of methyl laballenate in 40 ml. of acetic acid was added 15.5 g. of powdered potassium permanganate during 0.5 hr. After a short induction period, the reaction temperature increased to 45°. Reaction was continued 2.5 hr. at 40–50°, and then it was terminated in the usual manner. Steam distillation of the reaction mixture yielded 0.455 g. of a volatile fraction. The steam-nonvolatile phase, saturated with sodium chloride, was extracted with ethyl ether (ten 50-ml. portions). The combined ether extract was washed with 50 ml. of water and then dried over sodium sulfate. Removal of solvent *in vacuo* yielded 0.503 g. of mainly half-ester as determined by infrared spectroscopy. After methylation, g.l.p.c. analyses of the two fractions showed esters of lauric and glutaric acids as the major cleavage products. Equivalent chain length on the polar column for the dimethyl glutarate product was not identical with that reported by Miwa, *et al.*[24]; however, an admixture with authentic glutarate emerged as one peak. Infrared and n.m.r. spectra of the diester were identical with those of authentic dimethyl glutarate.

Hydrazine Partial Reduction of Ib.—Methyl laballenate (1.122 g.) was reduced partially with hydrazine as described previously.[28]

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After 16 hr., 1.042 g. of products composed of 52% of laballenic, 34% of monoenoic, and 13% of stearic acids was obtained. Methyl esters of this mixture were fractionated by c.c.d., as above, with the solvent system hexane–acetonitrile. The monoene fraction (0.329 g.) consisted of 96% of monoenes, 2% of laballenate, and 2% of palmitate: λ_{max} 183.5 mμ (ϵ 13,140). The infrared spectrum of the monoene mixture showed 19.9% of isolated *trans* unsaturation.[29]

Periodate–Permanganate Oxidation of IX.—Monoene mixture IX (0.161 g.) was oxidized with periodate–permanganate as described above. To resolve the cleavage products partially, an aqueous solution (10 ml.) of products was extracted with pentane–hexane (four 4-ml. portions). The combined extract was washed with water (two 3-ml. portions), dried over sodium sulfate, concentrated *in vacuo*, and esterified with diazomethane. The product (0.164 g., 167% yield on weight basis) was presumed to be methyl esters of monobasic acids. The aqueous solutions were reserved for subsequent isolation of dibasic acids. G.l.p.c. analyses of the monobasic acid methyl indicated 31% of methyl laurate, 43% of methyl tridecanoate, and 26% of unassigned material. The unidentified material may be polymeric material resulting from excess of diazomethane.[30] The two monobasic acids were resolved by preparative g.l.p.c. techniques. Collected fractions were saponified in the usual manner. *p*-Bromophenacyl esters were prepared by the procedure developed by Stodola.[31] Lauric acid (X, 0.003 g.) yielded, after recrystallization from 50% acetone, 0.002 g. of derivative melting at 65–67°; an admixture with authentic *p*-bromophenacyl laurate (m.p. 72.0–72.5°) melted at 66.5–71.0°. Tridecanoic acid (XI, 0.005 g.) yielded, after two recrystallizations from 70% ethanol, 0.003 g. of derivative melting at 66–68°. An admixture with authentic *p*-bromophenacyl tridecanoate (m.p. 71.5–72.0°) melted at 68.0–69.5°. X-Ray patterns of the two derivatives were identical with those of corresponding authentic specimens.

All aqueous fractions containing dibasic acids were combined, made alkaline, and concentrated on a rotating evaporator. The salt residue was acidified with hydrochloric acid and then extracted with ethyl ether. The ether extract, dried over sodium sulfate, was concentrated *in vacuo* to leave 0.038 g. of solid, m.p. 68–100°. Methyl esters were identified by g.l.p.c. analyses to be succinate (3%), glutarate (74%), and adipate (23%).

Chemical Reduction of Methyl Laballenate.—The ester group of methyl laballenate was reduced essentially by the procedure described by Hands and Bartley.[14] Methyl laballenate (1.00 g., 92.4% purity, 7.3% methyl oleate) was reduced with lithium aluminum hydride to give 0.923 g. The infrared spectrum showed hydroxyl, allene, and no carbonyl absorption (as distinctive characteristics). The *p*-toluenesulfonate, 1.517 g., was prepared and, in turn, reduced with lithium aluminum hydride to give 0.896 g. of a product containing some hydroxyl, demonstrated by infrared spectroscopy. The product was chromatographed on alumina to obtain 0.469 g. of nonhydroxylated components. G.l.p.c. analyses showed three major compounds: 7.6% of presumably *cis*-9-octadecene, 73.4% of 5,6-octadecadiene, and 13.9% of a material of higher molecular weight or greater polarity. Thin layer chromatography (t.l.c.) on silica gel G plates, with hexane as the developing solvent, indicated two major spots: R_f 0.93 and 0.71. These products were resolved on a silicic acid column activated 21 hr. at 120°. Fractions combined as indicated by t.l.c. yielded 0.294 g. of IV, λ_{max} 182.0 mμ (ϵ 19,060), and 0.060 g. of an unknown, λ_{max} 182.0 ($E^{1\%}_{1\,\mathrm{cm}}$ 706). Infrared spectra of each showed allene absorption at 5.1 μ. Allenic hydrocarbon IV, mass spectrum parent peak 250, was shown by n.m.r. to have six C-methyl protons, τ 9.12; 22 shielded methylene protons, τ 8.75; four allylic protons, τ 8.05; and two allenic protons, τ 5.03.[32]

Permanganate–Acetic Acid Oxidation of IV.—Allenic hydrocarbon IV was oxidized with permanganate in acetic acid as described above. G.l.p.c. analyses of free acid products showed 27% of valeric acid, 52% of lauric acid, 6% of pelargonic acid (from 9-octadecene), and 14% of miscellaneous degradation products together with residual acetic acid. *p*-Bromophenacyl esters of a portion of the oxidation mixture were prepared by the procedure described by Stodola.[31] The mixture of esters was separated on a column of activated (120°) silica gel. The laurate fraction recrystallized from 75% ethanol gave a solid that melted at 70.5–71.5°, both separately and in admixture with authentic *p*-bromophenacyl laurate (m.p. 72.0–72.5°).

Alkaline Isomerization of Laballenic Acid.—Methyl laballenate was refluxed 1 hr. under nitrogen with 10% sodium hydroxide. The infrared spectrum of esterified product was identical with that of the starting material. Reflux was continued 3 hr., but no apparent change was effected. The product was then heated at 170 ± 5° for 1 hr. in 10% potassium hydroxide–ethylene glycol solution. Esterified product and starting material had spectral differences at 8–10 μ. G.l.p.c. analyses indicated 3.1% of a monoacetylenic ester,[24] and two unassigned products (2.6 and 1.8%) (corrected according to starting composition).

Three portions (*ca.* 30 mg.) of methyl laballenate dissolved in 5 ml. of dimethyl sulfoxide containing 10% potassium *t*-butoxide were allowed to react under nitrogen at 27, 60–65, or 85–90°, respectively. The reactions were terminated by adding 20 ml. of water and acidifying with hydrochloric acid. The products were taken up in pentane–hexane in the usual manner. After removing the solvent *in vacuo*, the products had infrared spectra which showed the presence of allene (5.1 μ); however, the intensity of the absorption band had diminished by an inverse relationship with the reaction temperatures. The infrared spectra showed no evidence for acetylenic groups. The ultraviolet spectrum of the product obtained at 27° showed no maximum at 210–300 mμ; however, a slight inflection was apparent at 270 mμ. The product obtained at 60–65° had maxima at 221 and 230 mμ ($E^{1\%}_{1\,\mathrm{cm}}$ = 104 and 102, respectively), and the inflection at near 270 mμ was more pronounced. The product produced at 85–90° had one broad maximum at 230 mμ and a second maximum at 268 mμ ($E^{1\%}_{1\,\mathrm{cm}}$ = 197 and 78, respectively). G.l.p.c. analyses of the products obtained at 27, 60–65, and 85–90° indicated 49, 42, and 17%, respectively, of a component corresponding to monoacetylenic esters.[24] No structural assignments were made for other components in evidence.

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