*Biochem. J.* (1967) **105**, 1245
*Printed in Great Britain*



# An Octadecatrienoic Acid from *Lamium purpureum* L. Seed Oil Containing 5,6-Allenic and *trans*-16-Olefinic Unsaturation

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(*Received* 19 *July* 1967)

1. *Lamium purpureum* L. (Labiatae) seed oil contains 16% of a new acid characterized as (−)-octadeca-5,6-*trans*-16-trienoic acid (proposed trivial name 'lamenallenic acid') (Ia). The acid was isolated as its methyl ester by countercurrent distribution by using a combination of recycle–single withdrawal techniques. Methyl lamenallenate (Ib) is strongly laevorotatory. 2. The structure was established by infrared spectroscopy, nuclear-magnetic-resonance spectroscopy, quantitative hydrogenation and oxidative cleavage data of the original acid and of hydrazine partial reduction products. 3. Other unsaturated esters identified by their cleavage products were oleate, linoleate and linolenate. 4. A very small amount (less than 1%) of methyl laballenate [(−)-methyl octadeca-5,6-dienoate] was also isolated and identified.

Naturally occurring allenes have been found in a number of cases as fungal metabolites (Jones, 1960; Bonnett, Mallams, Tee & Weedon, 1966; Bew, Chapman, Jones, Lowe & Lowe, 1966). In all of these products of fungal origin, the allene grouping forms part of a conjugated system. Only two allenes have been isolated from higher plants. One occurs in the seed oil of *Leonotis nepetaefolia* (Labiatae) and was shown by Bagby, Smith & Wolff (1965) to be (−)-octadeca-5,6-dienoic acid (laballenic acid). Another allenic acid, 8-hydroxyocta-5,6-dienoic acid, was isolated from *Sapium sebiferum* seed oil (Sprecher, Maier, Barber & Holman, 1965). These allenes derived from seed oils differ from those elaborated by fungi in having an allene grouping that is not part of a conjugated system.

The present paper describes the isolation and characterization of a new allenic acid, to be called lamenallenic acid, from *Lamium purpureum* seed oil. Preliminary evidence indicating the presence of an allenic acid in this species was provided by Hagemann, Earle, Wolff & Barclay (1967).

## EXPERIMENTAL

Infrared (i.r.) spectra were determined with Perkin–Elmer Infracord model 137 and model 337 spectrophotometers, ultraviolet (u.v.) spectra were determined on hexane solutions with a Beckman DK-2A spectrophotometer, and the nuclear-magnetic-resonance (n.m.r.) spectrum was determined with a Varian A-60 spectrometer on a deuterochloroform solution containing tetramethylsilane as the internal reference. A Cary model 60 spectropolarimeter (2cm. cell) was used to determine the optical-rotatory-dispersion spectrum. Melting points, obtained with a Fisher–Johns block, are uncorrected. The method of Miwa, Mikolajczak, Earle & Wolff (1960) was used for analysis of methyl ester samples by gas–liquid partition chromatography. In the instances where free acids were analysed, a polyester (LAC-2 R-446) column was used and the components were identified by comparison of observed retention times with those of known compounds. Quantities measured by gas–liquid partition chromatography are reported as percentage areas.

Analytical thin-layer chromatography was done on silica gel G impregnated with $AgNO_3$ according to the method of DeVries & Jurriens (1963). The method described by Mikolajczak & Bagby (1965) was used for preparative thin-layer chromatographic separations except that plates 20cm. × 20cm. were used singly instead of in a sandwich arrangement.

Seed oil of *Lamium purpureum* was obtained by an overnight Soxhlet extraction of the ground seeds with light petroleum (b.p. 30–60°); the yield was 40·5%. Mixed methyl esters were prepared by refluxing the oil (under $N_2$ atmosphere) with 1% (v/v) $H_2SO_4$ in methanol for 3hr. and were recovered by the usual diethyl ether extraction method.

Isolation of the allenic methyl ester was achieved by countercurrent distribution of the mixed methyl esters (24·6g.) in a 200-tube automatic Craig–Post apparatus essentially as described by Bagby *et al.* (1965). Three transfers per tube were collected until a total of 700 transfers

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had been decanted. Solvent was evaporated *in vacuo* from selected tubes. The weight distribution is shown in Fig. 1. Analyses of appropriate fractions by gas–liquid partition chromatography and thin-layer chromatography indicated that methyl lamenallenate (Ib; Scheme 1) was present in transfers 361–468 along with large amounts of diene and triene esters. Transfers 361–468 were combined and subjected to a second countercurrent distribution with a combination of recycle and single withdrawal techniques (Craig, 1944). After 600 transfers (two recycles), fractions were collected as above until 1200 transfers had been made. The weight distribution plotted from this run is in Fig. 2.

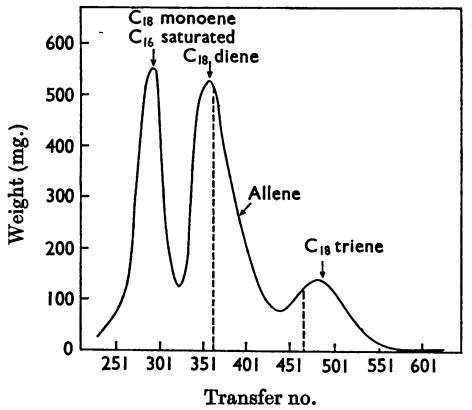


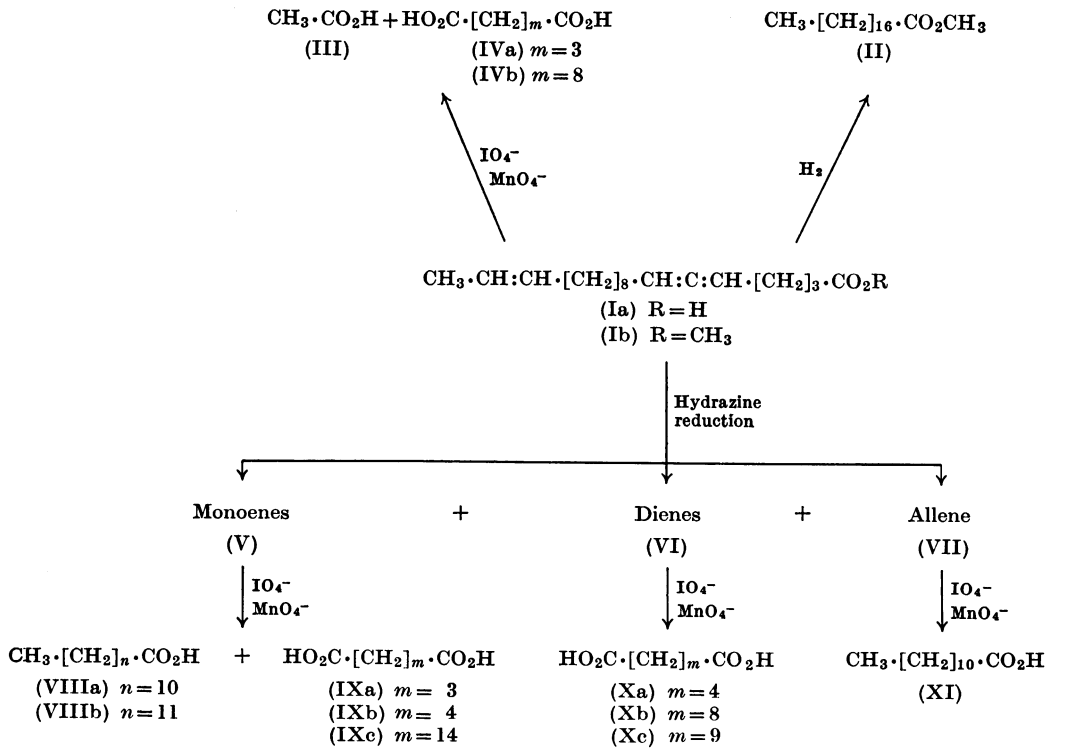

Fig. 1. Countercurrent distribution of *Lamium purpureum* methyl esters. Experimental details are given in the text.

Oxidative cleavage of the various ester fractions from countercurrent distribution and preparative thin-layer chromatography was accomplished with periodate–permanganate according to the procedure described by Rudloff (1956).

The conditions used for the hydrazine partial reduction of methyl lamenallenate (0·520g.) were the same as described by Mikolajczak & Bagby (1965) except that a reaction time of 35hr. was required. This length of time was necessary to yield sufficient quantities of all products for isolation by preparative thin-layer chromatography and identification by i.r. spectroscopy and oxidative cleavage. All reduction and cleavage products were esterified with 1% (v/v) $H_2SO_4$ in methanol.

## RESULTS

The i.r. spectrum of *L. purpureum* oil (2% in carbon disulphide) showed allene absorption (Wotiz & Mancuso, 1957) at 1950cm.$^{-1}$ and isolated *trans* absorption at 968cm.$^{-1}$. No significant maxima




Scheme 1.

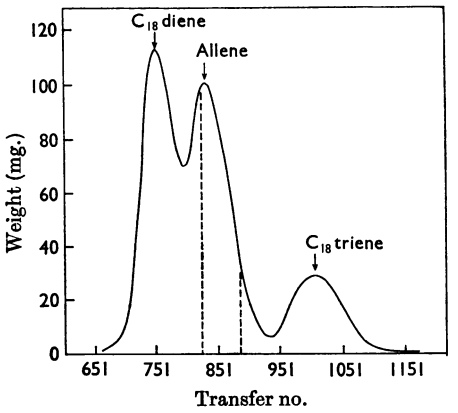



Fig. 2. Recycle countercurrent distribution of methyl lamenallenate concentrate. Experimental details are given in the text.

were observed in the u.v. spectrum between 210 and 320mμ. Transesterification of 25·4g. of oil yielded 24·7g. of mixed methyl esters comprised of the following as indicated by gas–liquid partition chromatography: palmitate, 11%; stearate, 2%; $C_{18}$ monoene, 25%; $C_{18}$ diene, 34%; and $C_{18}$ triene plus allenic ester, 28%. Quantitative i.r. analysis indicated that these mixed esters contained 16% of isolated *trans*-unsaturation (O'Connor, 1959).

Analysis of selected countercurrent fractions by gas–liquid partition chromatography, and by thin-layer chromatography on silver nitrate-impregnated silica gel G plates, showed that the peaks in Fig. 1 contained the components indicated. The material represented by the portion of the curve between the broken lines (6·8g.), after having been combined and recycled as described in the Experimental section, gave the weight distribution indicated in Fig. 2. After selected fractions were analysed by gas–liquid partition chromatography and thin-layer chromatography and appropriate fractions (indicated by the broken lines in Fig. 2) were combined, evaporation of solvent yielded a 1·6g. portion of 97·5%-pure methyl lamenallenate.

A sample of monoene contaminated with methyl palmitate was obtained by combining transfers 275–290, and a pure diene fraction resulted from combination of transfers 700–750. Similarly, the combination of transfers 480–530 from the first distribution (Fig. 1) yielded pure triene. No *trans* or allenic absorption was detected in the i.r. spectra of these fractions. Periodate–permanganate cleavage of these fractions and analysis of the products by gas–liquid partition chromatography before and after esterification demonstrated that the compounds were the expected methyl oleate, linoleate and linolenate. No unusual positional isomers were detected in any fraction.

The material in transfers 308–333 (Fig. 1) was shown by i.r. spectroscopy and by thin-layer chromatography on silver nitrate-impregnated silica to contain a small amount of an allenic compound. This compound (less than 1% of original ester mixture) was isolated by preparative thin-layer chromatography and was identified as methyl octadeca-5,6-dienoate (Bagby *et al.* 1965) by oxidative cleavage.

Quantitative i.r. analysis (O'Connor, 1959) of methyl lamenallenate (Ib) established that it contained one isolated *trans*-double bond per molecule and also showed strong allene absorption at 1950cm.$^{-1}$. The u.v. spectrum had no observable maxima between 210 and 350mμ (0·5% in hexane). At 25° compound (Ib) gave an optical-rotatory-dispersion spectrum (*c* 0·28 in ethanol) with the following characteristics: $[\alpha]_{589}$, −50°; $[\alpha]_{400}$, −141°; $[\alpha]_{300}$, −307°; $[\alpha]_{260}$ (trough), −404°; $[\alpha]_{235}$, −36°.

Compound (Ib) (0·0191g.) in ethanol readily absorbed 3·1 mole equivalents of hydrogen in the presence of platinum catalyst to yield 0·0184g. of compound (II) (methyl stearate), m.p. 38–39°, mixed m.p. with authentic methyl stearate, 38–39°. Analysis of compound (II) by gas–liquid partition chromatography indicated 99·8% methyl stearate.

Compound (Ia) (from saponification of compound Ib) yielded a *p*-bromophenacyl ester, m.p. 40·5–41·5°, recrystallized from 80% ethanol, when treated in the manner described by Stodola (1963) (Found: C, 65·7; H, 7·65; Br, 16·6. Calc. for $C_{26}H_{35}BrO_3$: C, 65·68; H, 7·42; Br, 16·81).

Acetic acid (III) was the only monobasic acid detected by gas–liquid partition chromatography of the oxidative cleavage fragments from compound (Ib); the conditions used were appropriate to detect free monobasic acids up to dodecanoic acid. A portion of the cleavage products was esterified. Analysis of the resulting methyl esters by gas–liquid partition chromatography revealed that compounds (IVa) and (IVb), methyl glutarate (22%) and methyl decanedioate (74%), were the major products. These results pinpoint one of the unsaturated centres at C-16, but they do not show whether it is the allene grouping or the *trans*-double bond.

Analysis of the esterified hydrazine reduction products by gas–liquid partition chromatography showed the following types of materials: $C_{18}$ saturated, 17%; $C_{18}$ monoenes (V), 26%; $C_{18}$ dienes (VI and VII), 45%; unchanged compound (Ib), 12%. Preparative thin-layer chromatography on silica gel G impregnated with silver nitrate separated this ester mixture into six fractions according to degree and type of unsaturation.

Fraction 1, $R_F$ 0·92, was 93% methyl stearate.

The i.r. spectrum of fraction 2, $R_F$ 0·88, indicated strong allene (1950cm.$^{-1}$) absorption and a trace of *trans* absorption. This fraction contained 90% of compound (VII), methyl laballenate, that resulted from reduction of the isolated *trans*-double bond in compound (Ib). Cleavage of compound (VII) and analysis of the products by gas–liquid partition chromatography before and after esterification showed that dodecanoic acid (XI) was the only monobasic acid fragment. The dibasic acid fragment, glutaric acid, accounted for about 5% of the cleavage products. Positive identification of compound (XI), however, demonstrates that the allene grouping is located in the 5,6-position in compound (VII) and also in compound (I).

Fraction 3, $R_F$ 0·83, was shown by i.r. spectroscopy, gas–liquid partition chromatography and oxidative cleavage to be a mixture of methyl octadec-*trans*-5-enoate and methyl octadec-*trans*-6-enoate (45%), methyl octadec-*trans*-16-enoate (23%) and 29% of the allene, methyl laballenate.

Cleavage data established that the *cis*-monoenes (fraction 5, $R_F$ 0·72) were methyl octadec-5-enoate and methyl octadec-6-enoate. All the cleavage fragments (VIII and IX) from both the *cis*- and *trans*-monoene fractions (V) are depicted in Scheme 1.

The dienes (VI) resulting from reduction of one bond of the allene grouping in compound (Ib) appeared as two bands, $R_F$ 0·50 and 0·42, which were recovered together as fraction 6. Since both dienes contain a *trans*-16-double bond, the only monobasic cleavage fragment would be acetic acid, but no attempt was made to identify it because of the small amount of material available. The dibasic cleavage fragments (Xa, Xb and Xc) indicate that compound (VI) was a mixture of methyl octadeca-6,16-dienoate and methyl octadeca-5,16-dienoate. Glutaric acid, which should have been a major component, represented less than 5% of the cleavage fragments.

Fraction 4, $R_F$ 0·78, from the preparative thin-layer chromatographic separation was identified as unchanged compound (I). Overall recovery of the reduction products was 75%.

The positive identification of the methyl esters of octadeca-5,6-, -5,16- and -6,16-dienoic acids and octadec-5-, -6- and -*trans*-16-enoic acids in the hydrazine reduction mixture shows that lamenallenic acid is octadeca-5,6-*trans*-16-trienoic acid.

The n.m.r. spectrum of methyl lamenallenate, reproduced in Fig. 3, is consistent with the structure (Ib) shown in Scheme 1. The 16,17-double bond causes the signal due to terminal methyl protons to appear at 8·53$\tau$ instead of at 9·1$\tau$, where it is found for compounds with the methyl group attached to a chain of at least two methylene

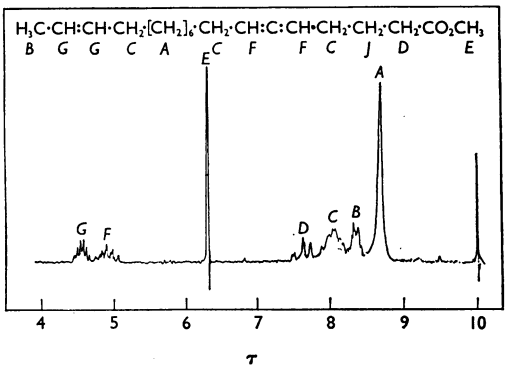



Fig. 3. Nuclear-magnetic-resonance spectrum of methyl lamenallenate. Experimental details are given in the text. The signal due to protons labelled *J* is obscured by signal *B* or *C*.

groups. Signals *B*, *C* and *D* have a combined area equivalent to 13 protons. Since the protons on C-3 of methyl glutarate give a signal centred at 8·01$\tau$, it is reasonable to assume that the signal due to C-3 protons (*J*) of methyl lamenallenate is obscured by signal *C* in Fig. 3. Areas of all other signals are equivalent to the number of corresponding protons shown in the formula.

## DISCUSSION

Methyl lamenallenate appeared in the first countercurrent distribution weight curve (Fig. 1) where expected. Methyl laballenate (Bagby *et al.* 1965) overlapped the monoene in the hexane/acetonitrile system. Methyl lamenallenate has an additional double bond and therefore should appear near the diene peak. This proved to be the case. The amount of overlap between the diene peak and the lamenallenate peak was such that it was necessary to employ the recycle technique to obtain sufficient pure methyl lamenallenate for characterization.

On a polyester gas–liquid chromatographic column, methyl lamenallenate emerges with methyl linolenate; hence this method alone was not sufficient to ascertain the purity of the various countercurrent fractions. Analysis by thin-layer chromatography on silica gel G impregnated with silver nitrate was necessary to detect the presence of linolenate impurities. On this adsorbent, compound (Ib) migrated between the *cis*- and *trans*-monoenes but slightly overlapped the *cis*-isomer. The allene was readily detected because it charred more rapidly than the other components. This same general procedure was used to analyse the octadeca-5,6-dienoate formed during the hydrazine reduction because it emerges from the polyester gas–liquid

chromatographic column with the normal $C_{18}$ dienes.

Hydrazine reductions have been applied with success to a number of different types of compounds by Aylward & Rao (1956), Scholfield, Jones, Nowakowska, Selke & Dutton (1961), Bagby, Smith, Mikolajczak & Wolff (1962), Schilling (1963), Bagby *et al.* (1965) and Mikolajczak & Bagby (1965). Application of the hydrazine reduction to an allenic grouping (Bagby *et al.* 1965) demonstrated that both *cis*- and *trans*-monoenes are formed by partial reduction of this grouping. This phenomenon was also observed in the hydrazine partial reduction of the allene grouping in methyl lamenallenate. The relative amount of each geometric isomer formed was not determined. The *trans*-16-double bond of compound (Ib) apparently is reduced slightly faster than the allenic bonds because at no point in the reaction is the concentration of the *trans*-16-monoene as high as that of the 5-monoene or 6-monoene.

Preparative thin-layer chromatography was the method chosen to separate the partial reduction mixture because of the number and kinds of components involved. This method gave samples of allene, monoenes and dienes sufficiently pure for cleavage work, whereas countercurrent distribution with the solvent system ordinarily used would have resulted in much overlap.

Bagby *et al.* (1965) were unable to detect glutaric acid in the periodate–permanganate cleavage products from methyl laballenate. The same difficulty was experienced in this work with cleavage of the octadeca-5,6- and -5,16-dienoic acids. Cleavage of methyl lamenallenate and the *cis*- and *trans*-5-monoenes, however, gave nearly theoretical recoveries of glutaric acid. The reason for this anomolous behaviour is unexplained.

Lamenallenic acid [(−)-octadeca-5,6-*trans*-16-trienoic acid] is the second member of the new class of $C_{18}$ allenic fatty acids containing non-conjugated unsaturation. The presence of the *trans*-16-double bond makes this acid unique among those isolated from higher plants, although octadec-*trans*-16-enoic acid has been reported in butterfat (Backderf & Brown, 1958; Hansen & Cooke, 1961) and in other animal lipids (Hansen, 1963).

Landor & Punja (1966) synthesized (−)-laballenic acid and found that it has the *R*-configuration according to the designation established by Cahn, Ingold & Prelog (1956) and Cahn (1964). The specific rotation reported for the synthetic compound has the same sign but only one-tenth the magnitude of the rotation of the naturally occurring acid (Bagby *et al.* 1965). Since lamenallenic acid also is laevorotatory and since its structure differs from that of laballenic acid only by a double bond far removed from the allene grouping, one can reasonably expect that it, too, has the *R*-configuration.

We thank Dr Q. Jones, Crops Research Division, U.S. Department of Agriculture, Beltsville, Md., U.S.A., for supplying *Lamium* seeds, Mr J. W. Hagemann for gas–liquid chromatographic analyses, Mr L. W. Tjarks for the nuclear-magnetic-resonance spectrum, and Mrs C. E. McGrew and Mrs B. R. Heaton for microanalyses. The mention of firm names or trade products in this paper does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

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