(95% C_2H_5OH) 232 nm (ε 13,800) and 300 (5100); ir (KBr) 2.95, 3.35, 3.40, 3.50, 5.72 (sh), 5.81, 5.90, and 6.22 μ; mass spectrum *m/e* 394 (M^+), 376, 358, 348, 347, 257.

Anal. Calcd for $C_{24}H_{26}O_5$: 394.1780. Found: 394.1782.

Bersenogenin 14,16-Phenylboronate (10).—To a solution of bersenogenin (**3**, 19.5 mg) in dry acetone was added phenylboronic acid (5.74 mg) and the solution was allowed to stand at room temperature for 7 min. On addition of hexane (1 ml) crystals formed. The product was collected by filtration and recrystallized from chloroform–hexane to give the cyclic boronic ester (**10**, 13 mg) as needles: mp 221–224°; uv max (CH_3OH) end absorption, 298 nm (ε 6100); mass spectrum *m/e* 423 ($M - C_6H_5$), 405, 377.

Anal. Calcd for $C_{30}H_{33}O_6B$: C, 72.00; H, 6.60; B, 2.20. Found: C, 71.84; H, 6.73; B, 2.12.

Registry No.—**1**, 30344-95-7; **2**, 30344-96-8; **3**, 30344-97-9; **5**, 510-62-3; **6**, 30344-99-1; **7**, 30345-00-7, **8**, 30345-01-8; **9**, 30345-02-9; **10**, 30345-03-0; **11**, 465-90-7; **12**, 23044-69-1; **13**, 23044-67-9; **14**, 23044-72-6.

Identification and Synthesis of the Four Compounds Comprising the Boll Weevil Sex Attractant[1a]

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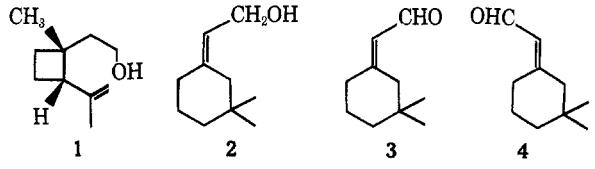
Four terpenoid compounds, (+)-*cis*-2-isopropenyl-1-methylcyclobutaneethanol, (**1**), *Z*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneethanol (**2**), *Z*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde (**3**), and *E*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde (**4**), were identified as the components of the male sex pheromone of the boll weevil. The synthesis and structural assignments of the four compounds are also reported.

Insect sex attractants (pheromones) are currently of considerable interest since they may provide a generally nontoxic method of surveying and controlling insect populations.[2] The growing concern over the environmental pollution and ecological imbalance caused by insecticides has further stimulated interest in this area.

A pheromone complex emitted by live male boll weevils (*Anthonomus grandis* Boheman) elicits a response by female weevils in laboratory assays.[3] The volatile components of this complex and other compounds were obtained by steam distillation of the crude extracts of 4.5 million weevils and 54.7 kg of weevil feces. The concentrated dichloromethane extract of the steam distillate mimicked the attractiveness of live males in laboratory tests.[4]

We have now isolated, identified, and synthesized four terpenoid compounds (**1**, **2**, **3**, **4**) which account for all of the attractancy of live male weevils. The response by females to mixtures of the synthetic compounds is identical with their response to corresponding mixtures of the natural compounds.



1 2 3 4

The extract of the steam distillate from weevils and their feces was fractionated by column chromatography on Carbowax 20M coated[5] silica gel. None of the individual fractions from this column were attractive to females, but the combination of two of the fractions was as active as the original distillate. Each of these two fractions was then separately fractionated on a column containing Adsorbosil-CABN (25% $AgNO_3$ on silica gel).

Various recombinations of all the fractions from both $AgNO_3$–silica gel columns yielded two fractions, one from each column, that were attractive together but totally unattractive separately. Each of these latter two active fractions was then fractionated by glpc on Carbowax 4000 and SE-30. Three components were collected which were attractive when all three were combined but which were unattractive individually or in pairs. Rechromatography on Carbowax 4000, SE-30, and a 50-ft support coated open tubular (SCOT) column showed two of these components to be pure (**1**, **2**) and the third to consist of two compounds (**3**, **4**). Concentrations of compounds **1**, **2**, **3**, and **4** in fecal material, determined by glpc, were 0.76, 0.57, 0.06, and 0.06, respectively. Concentrations in weevils were about tenfold less. Compound **1** was identified as (+)-*cis*-2-isopropenyl-1-methylcyclobutaneethanol on the basis of mass, ir, and nmr spectra.[6] The cis configuration was assigned by comparison with the nmr spectrum of the synthetic cis isomer (*vide infra*). The optical rotation was measured on 11 mg of the pure natural compound. The specific rotation was estimated to be about +50° (±10°).

Scheme I outlines the synthesis of *cis*-2-isopropenyl-1-methycyclobutaneethanol. The photocycloaddition of isoprene and 3-buten-2-one produced several products, many of them in greater yield than the desired isomer of 2-methyl-2-vinylcyclobutyl methyl ketone **5**. Compounds **7**, **8**, **9**, **10**, **11**, and **12** were tentatively iden-

(1) (a) Taken in part from the Ph.D. thesis of J. H. Tumlinson, Mississippi State University, State College, Miss., June 1969. (b) Authors to whom inquiries should be addressed at the Boll Weevil Research Laboratory, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, State College, Miss. 39762.

(2) M. Jacobson, "Insect Sex Attractants," Interscience, New York, N. Y., 1965.

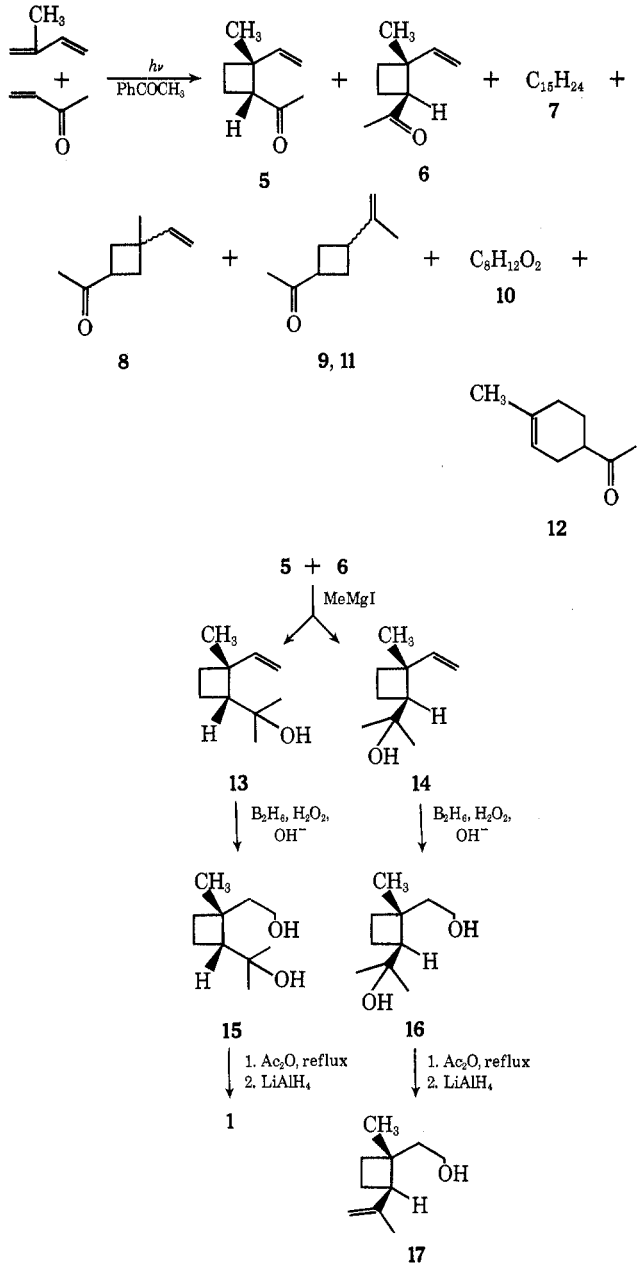
(3) (a) W. H. Cross and H. C. Mitchell, *J. Econ. Entomol.*, **59**, 1503 (1966); (b) D. D. Hardee, E. B. Mitchell, and P. M. Huddleston, *ibid.*, **60**, 169 (1967); **60**, 1221 (1967).

(4) J. H. Tumlinson, D. D. Hardee, J. P. Minyard, A. C. Thompson, R. T. Gast, and P. A. Hedin, *ibid.*, **61**, 470 (1968).

(5) Mention of a proprietary product in this paper does not constitute an endorsement of this product by the U. S. Department of Agriculture.

(6) A preliminary report of this work discussing the isolation and identification was published by J. H. Tumlinson, D. D. Hardee, R. C. Gueldner, A. C. Thompson, P. A. Hedin, and J. P. Minyard, *Science*, **166**, 1010 (1969).

SCHEME I



tified on the basis of spectral data and elemental analyses. Irradiation with the Vycor filter for 65 hr produced the same amounts of products **5–12** that were produced in 130 hr with the Pyrex filter but the amount of polymer produced with the Vycor filter was about tenfold greater. The major reaction product, 4-methyl-3-cyclohexenyl methyl ketone (**12**), resulted from the thermal Diels–Alder addition of isoprene and 3-buten-2-one.[7a] The yield of this side reaction product was minimized by low reaction temperatures.

Because it was impossible to separate the *cis*- and *trans*-2-methyl-2-vinylcyclobutyl methyl ketones by any chromatographic methods available, the Grignard addition was carried out on the mixture. The resulting tertiary alcohols (**13**, **14**) were readily separated by glpc on Carbowax 4000 and were purified in this way. Since the Grignard addition produced quantitative yields of the alcohols, as determined by glpc, it was calculated that the *cis*- and *trans*-2-methyl-2-vinylcyclobutyl methyl ketones were each produced in 1.2% yield in the photocycloaddition. At this point, however, positive assignment of configuration to the ketones and alcohols was impossible.

Subsequently, the two α,α,2-trimethyl-2-vinylcyclobutanemethanols (**13**, **14**) were hydroborated separately to produce the two diols (**15**, **16**).[7b] The diols were each acetylated and the esters pyrolyzed in a single step. The resulting unsaturated esters were each reduced with $LiAlH_4$ to the alcohols, *cis*- and *trans*-2-isopropenyl-1-methylcyclobutaneethanols (**1** and **17**), respectively.

The nmr spectra of *cis*- and *trans*-2-isopropenyl-1-methylcyclobutaneethanol are presented in the microfilm. The significant feature which allowed assignment of the trans configuration to **17** was the upfield shift of the methyl singlet from 1.22 in the cis to 0.92 in the trans due to the diamagnetic shielding of the spatially adjacent olefinic π electrons in the trans isomer. Similarly, the methylene (CH_2CH_2OH) was shifted downfield in the trans isomer since it is not adjacent to the olefinic bond in the isopropenyl group as in the cis compound. The naturally occurring compound and the synthetic compound **1** assigned the cis configuration on the basis of its nmr spectrum were identical in nmr, ir, and mass spectra and in biological activity.[8]

The structure of *Z*-3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol[9] (**2**) was elucidated on the basis of its mass, nmr, and ir spectra.[6]

Reduction of compound **2** on palladium catalyst immediately ahead of a Carbowax 20M SCOT column[10] gave a compound with a parent mass of 156 and other major fragments in the spectrum two mass units higher than the corresponding fragments of compound **2**, indicating one unsaturation.

The OH stretch at 3610 cm^{-1}, less hydrogen bonded than compound **1**, the upfield shift of a one-proton signal (1.90) in the nmr spectrum on dilution, and removal of this signal on addition of TCAIC ($Cl_3CCONCO$) confirmed that the compound was an alcohol. The cis or *Z* configuration about the double bond was assigned by comparison of the nmr spectra of the cis *Z* (**19**) and trans *E* (**20**) synthetic ester precursors (*vide infra*).

Further proof of structure was obtained by microozonolysis.[11] One major component was obtained that was identical in nmr, mass spectrum, and glpc behavior with that of 3,3-dimethylcyclohexanone.

Scheme II shows the synthesis of *Z*-3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol (**2**). The 3,3-dimethylcyclohexanone was produced from 3-methyl-2-cyclohexen-1-one by the method of Büchi, *et al.*[12] The addition of ethyl bromoacetate (Reformatsky) to the 3,3-dimethyl-

(7) (a) E. F. Lutz and G. M. Bailey, *J. Amer. Chem. Soc.*, **86**, 3899 (1964); (b) H. C. Brown and B. C. Subba Rao, *ibid.*, **81**, 6428 (1959).

(8) The ir, nmr, and mass spectra for compounds **1** and **2** and the mass spectra for **3** and **4** will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Reprint Department, ACS Publications, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to author, title of article, volume, and page number. Remit $3.00 for photocopy or $2.00 for microfiche.

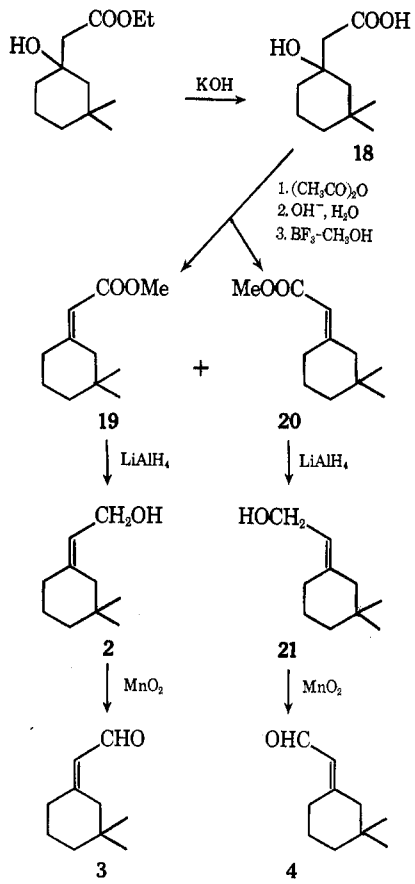
(9) The configurational descriptors *Z* and *E* are used in place of cis and trans, respectively. See "IUPAC Tentative Rules for the Nomenclature of Organic Chemistry, Section E. Fundamental Stereochemistry," *J. Org. Chem.*, **35**, 2851 (1970).

(10) M. Beroza and R. Sarmiento, *Anal. Chem.*, **38**, 1042 (1966).

(11) M. Beroza and B. A. Bierl, *ibid.*, **38**, 1976 (1966); **39**, 1131 (1967).

(12) G. Büchi, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, **31**, 241 (1948).

SCHEME II

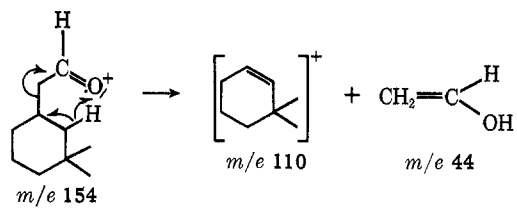


cyclohexanone[13] and then saponification yielded 1-hydroxy-3,3-dimethylcyclohexaneacetic acid. The hydroxy acid was dehydrated with acetic anhydride,[14] and the unsaturated acids were esterified with BF_3–CH_3OH. The cis and trans unsaturated esters were separated by distillation, further purified by glpc on a preparative SE-30 column, and reduced to the respective cis and trans alcohols with $LiAlH_4$.

Assignment of the *Z* (cis) and *E* (trans) configurations was made by comparing the nmr spectra of the *Z* and *E* esters. The *Z* ester spectrum showed a singlet at 5.55 ($MeOOCCH{=}CRCH_2CR'R_2''$) and a singlet at 2.61 ($MeOOCCH{=}CRCH_2CR'R''$). This latter signal indicated a considerable paramagnetic deshielding of the methylene at the 2 position on the ring by the spatially adjacent carbonyl group in the *Z* ester. The *E* ester, on the other hand, had a singlet at 5.42 (1 H, olefinic) produced by the more shielded proton cis to the geminal methyls. A triplet at 2.73 (2 H) occurs because the ring methylene at the 6 position is deshielded by the adjacent cis carbomethoxy group and split by the adjacent ring methylene. The ring methylene at the 2 position of *E* produced a singlet at 1.93, well upfield of the analogous methylene (2.12) at the 6 position in the *Z* ester.[15] The natural compound was identical in all respects including insect attractancy with the synthetic 3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol assigned the *Z* configuration.

(13) C. A. Vodoz and H. Schinz, *Helv. Chim. Acta*, **33**, 1040 (1950).
(14) G. H. Elliott and R. P. Linstead, *J. Chem. Soc.*, 776 (1938).
(15) These assignments are consistent with those made by R. H. Bible, Jr., "Interpretation of NMR Spectra, an Empirical Approach," Plenum Press, New York, N. Y., 1968, pp 17, 18.

When compounds **3** and **4** were eluted as a single peak from a glpc column into 2,4-dinitrophenylhydrazine reagent on a tlc plate,[16] a derivative was produced which had an R_f similar to that of standard terpene carbonyls. The mass spectra of compounds **3** and **4** were nearly identical with each other and similar to compound **2**. The parent peak had a *m/e* of 152 in both cases, appropriate for a monocyclic terpene aldehyde or ketone with one unsaturation. Reduction of compounds **3** and **4** at the inlet of a gas chromatograph[10] produced only one peak with a parent mass of 154, which confirmed the single unsaturation. The base peak in the spectrum of saturated **3** and **4**, *m/e* 110, suggests a facile loss of the elements of acetaldehyde, and such a rearrangement peak suggests a $-CH_2CH{=}O$ side chain which might easily cleave by a cyclic rearrangement process analogous to the following.



On the basis of these data, structures **3** (*Z*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde) and **4** (*E*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde) were postulated.

Postulated structures **3** and **4** were quickly confirmed by synthesis as follows. Compound **2** and its *E* isomer were readily converted to the *Z* and *E* aldehydes **3** and **4**, respectively, by stereospecific oxidation with active MnO.[17] The mass spectra, glpc behavior, and biological activity of the natural compounds **3** and **4** were identical with those of the synthetic compounds assigned the *Z* and E configurations, respectively.

Synthetic compounds (**1**, **2**, **3**, **4**) are as attractive to female boll weevils as the natural compounds when they are combined in the proper proportions. To our knowledge, none of these four compounds has been found previously in natural products. Compounds **2**, **3**, and **4** have been synthesized but not stereochemically characterized. It seems likely that the two 3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanols and the corresponding aldehydes were obtained enroute to the synthesis of 3-methyl-4-allocyclogeranyl-2-butenoic acid and related compounds.[13,18,19] More recently, Corey[20] has synthesized *trans*-2-isopropenyl-1-methylcyclobutaneethanol, which appears identical in ir and nmr spectra with our trans compound **17**, and Zurflüh,[21] *et al.*, have reported the synthesis of *cis*-2-isopropenyl-1-methylcyclobutaneethanol which is identical in nmr spectrum and biological activity with our cis isomer. Our trans isomer **17** which may have contained a trace of the cis isomer was active only at 100- to 200-fold greater concentrations.

Other routes of synthesis are being investigated for all four compounds. In particular the yield of **1** from

(16) J. H. Tumlinson, J. P. Minyard, P. A. Hedin, and A. C. Thompson, *J. Chromatogr.*, **29**, 80 (1967).
(17) E. J. Corey, N. W. Gilman, and B. E. Ganem, *J. Amer. Chem. Soc.*, **90**, 5616 (1968).
(18) Cl. Daessle and H. Schinz, *Helv. Chim. Acta*, **39**, 2118 (1956).
(19) Ch. A. Vodz and H. Schinz, *ibid.*, **33**, 1321 (1950).
(20) E. J. Corey, private communication.
(21) R. L. Zurflüh, L. L. Duham, Virginia L. Spain, and J. B. Siddall, *J. Amer. Chem. Soc.*, **92**, 425 (1970).

the method described in the present investigation is too low for use in large-scale field studies. Preliminary field tests indicate that the combination of the four compounds is attractive in the field as well as in the laboratory.

Experimental Section

General.—All elemental analyses were carried out by Galbraith Laboratories, Knoxville, Tenn. Spectrometers used were the Varian A-60 (nmr), the Perkin-Elmer Model 521 (ir), and the Perkin-Elmer Model No. 270 mass spectrometer which includes a gas chromatographic inlet system. All nmr and ir spectra were run in CCl_4 unless otherwise indicated. All nmr peaks are reported as δ in parts per million and coupling constants as J in hertz. Gas chromatographs used for analytical work were the Aerograph Model A-95-P_3, the Aerograph Model 600C, and the Barber-Colman Model 5000 equipped with an effluent splitter; for preparative glpc, the Aerograph Model A-700 and the Aerograph Model 90-P were used. The procedure used for laboratory assays was that of Hardee, *et al.*[3b] All fractionations were monitored by laboratory bioassay, and samples submitted for assay were all provided in dichloromethane solution. The active compounds were assayed individually and in various combinations to determine the mixture which would give optimum response.

Column Chromatography.—Silica gel coated with Carbowax 20M in the manner of Kuglar and Kováts[22] was used in the initial fractionation to avoid isomerization of the notoriously labile terpenoids and the steam distillate extracts of 39.2 kg of fecal material and 42.5 kg of weevils was chromatographed in this way. The fractions from the initial column were subsequently chromatographed on Adsorbosil-CABN (25% silver nitrate treated silica gel, Applied Science Labs), which had been washed successively with acetone–ether (50:50), ether, and pentane before use.

Gas–Liquid Partition Chromatography.—The following columns were used for glpc: Column A, Carbowax 4000, 6 ft × 1/8 in., 28.5% on 60–80 Gas-Chrom P, 135° column temperature with 30 psi nitrogen (approximate flow rate was 20 cm³/min); column B, SE-30, 20 ft × 1/8 in., 10% 60–80 Gas-Chrom Q, 160° column temperature with 64 psi nitrogen (50 cm³/min); column C, Carbowax 4000, 20 ft × 1/8 in., 30% on 60–80 Gas-Chrom P, 140° column temperature with 27 psi nitrogen (24 cm³/min); column D, Carbowax 20M, 50 ft, support coated open tubular (SCOT), 110° column temperature with 2 psi helium (2–3 cm³/min); column E, SE-30, 20 ft × 3/8 in., 10% 60–80 Gas-Chrom P, 160° column temperature with 40 psi helium (200 cm³/min); and column F, Carbowax 4000, 20 ft × 3/8 in., 30% on Gas-Chrom P, 170° column temperature with 60 psi helium (190 cm³/min).

Fractions from column chromatography were initially separated on column A. Components collected from this column were then injected onto column B and column E. Peaks were collected from both Carbowax 4000 and SE-30 columns for assay by bubbling through dichloromethane and for spectral studies by bubbling through carbon tetrachloride. Column D was used in the gas chromatographic inlet system to the mass spectrometer.

Sample compounds were reduced just before introduction into the mass spectrometer by placing a 0.25-in. Swagelok connector containing about 1 cm of neutral palladium catalyst in the oven between the gas chromatographic injector and the SCOT column (D). The fitting containing the palladium was conditioned by sweeping with hydrogen for 30 min at 150°.[10]

Derivatization.—2,4-Dinitrophenylhydrazine (DNPH) (5 g) was dissolved in 60 ml of 85% H_3PO_4 and 40 ml of 95% ethanol. Tlc plates (250-μ adsorbent depth) were prepared on 20 × 20 cm × 3 mm glass with silica gel G (SGG). The standard carbonyls, methone, pulegone, and carvone, were chromatographed on column A, and the effluent was allowed to flow into a drop of the DNPH reagent on a SGG plate.[16] The derivatives formed in this way were developed on the plate with benzene and petroleum ether (4:1) to a height of 10 cm. Natural compounds **3** and **4** were derivatized in the same way, and the plate was developed in the same solvent. The sample derivatives and the standard derivatives were scraped from the initial plate, eluted with dichloromethane, and respotted side by side on a second SGG plate which was then developed in benzene and petroleum ether. The R_f vaues of the DNPH's of the standards and of **3** and **4** were about the same.

Microozonolysis.—The natural Z-3,3-dimethyl-$\Delta^{1,8}$-cyclohexaneethanol (**2**) was ozonized with a Supelco Microozonizer.[11] About 15 mg of **2**, glpc pure, in 200 μl of carbon disulfide was ozonized for 5 min. Reinjection on a SE-30 glpc column indicated that nearly all the sample had reacted. This ozonolysis was repeated 15 times with 200 μl of solution each time. The reaction mixture was chromatographed on SE-30, and the only major peak was collected by bubbling through CCl_4. The glpc retention time, nmr, and mass spectrum of this compound were identical with those of 3,3-dimethylcyclohexanone.

Photocycloaddition.—A mixture of 172 g (2.53 mol) of isoprene, 108 g (1.80 mol) of 3-buten-2-one, and 20 g (0.17 mol) of acetophenone was irradiated with a 450-W Hanovia mercury vapor lamp through a Pyrex filter for 130 hr, through a Corex filter for 88 hr, or through a Vycor filter for 65 hr. Analysis of the reaction products by glpc (column C) revealed seven major components. The yields of each were calculated by measurement of peak areas relative to an internal standard. All components were collected, on elution, in carbon tetrachloride for spectral analysis and neat by condensation in glass tubes for elemental analysis.

Component 1 (retention time 7.8 min), 2.4% yield, contained two compounds, a sesquiterpene **8**, and a ketone, $C_9H_{14}O$, which showed as a shoulder on the backside of component 1.

Anal. Calcd for $C_{15}H_{24}$: C, 88.16; H, 11.83. Found: C, 88.19; H, 12.12.

Component 2 (retention time 10.7 min), 2.4% yield, contained both *cis*- and *trans*-2-methyl-2-vinylcyclobutyl methyl ketone (**5** and **6**, respectively) which were not separable by any means available. Component 2 showed the following spectral characteristics: ir 1700 (C=O), 1630 (C=C), 910 cm^{-1} (CH=CH_2); nmr 1.11 and 1.34 (s, $CH_3CRR'R''$), 1.7–2.7 (complex envelope), 1.99 and 2.08 (s, CH_3C=O), 3.13 and 3.28 (t, overlapping, $CH_3COCHRR'$), 4.8–5.4 (m, CH=CH_2), 6.07 (m, J_{cis} = 10, J_{trans} = 18, CH=CH_2), 6.26 (m, J_{cis} = 10.5 J_{trans} = 17.5, CH=CH_2).

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.08; H, 10.14.

Component 3 (retention time 11.6 min), 3.3% yield, contained 3-methyl-3-vinylcyclobutyl methyl ketone (**7**) and had the following spectral characteristics: ir nearly identical with ir of component **2**; nmr 1.28 (s, 3, $CHCRR'R''$), 1.6–2.9 (complex m, 4, two CH_2), 1.99 (s, 3, CH_3C=O), 3.19 (t, 1, J = 8.5, CH_3CO-$CHRR'$), 4.88 (m, J_{trans} = 17, J_{cis} = 10, 2, CH=CH_2), 5.92 (m, 1, J_{trans} = 17, J_{cis} = 10, CH=CH_2).

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.21; H, 10.27.

Component 4 (retention time 13.6 min), 1.5% yield, was identified as 3-isopropenylcyclobutyl methyl ketone (**9**) and had the following spectral characteristics: ir 1705 (C=O), 1370 and 1350 (vinyl methyl and ketone methyl), 1643 (C=C), 884 cm^{-1} (CH=CH_2); nmr 1.58 (broad s, 3, CH_3(R)C=CH_2), 1.7–2.95 (complex m, 4, two CH_2), 1.88 (s, 3, CH_3C=O), 2.92 (two t, nearly superimposed, 2, H_2C=C(CH_3)CHRR′ and CH_3CO-CHRR′), 4.57 (broad s, 2, R(CH_3)C=CH_2).

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.22; H, 10.43.

Component 5 (retention time, 15.3 min), 1.4% yield, was identified as 3,4-dihydro-6-methyl-2*H*-pyran-2-yl methyl ketone (**10**), a dimer of methyl vinyl ketone, and was also present in the starting material.[23]

Anal. Calcd for $C_8H_{12}O_2$: C, 68.54; H, 8.63. Found: C, 68.35; H, 8.79.

Component 6 (retention time 17.7 min), 0.8% yield, was identified as 3-isopropenylcyclobutyl methyl ketone (an isomer of component **9**) (**11**) and had the following spectral characteristics: ir 1705 (C=O), 1640 (C=C), 1360 (broad, two CH_3), 888 cm^{-1} (C=CH_2); nmr 1.62 (broad s, 3, CH_3(R)C=CH_2), 1.8–3.2 (complex m, 6, two CH_2, H_2C=C(CH_3)CHRR′ and CH_3CO-CHRR′), 1.94 (s, 3, CH_3C=O), 4.60 (broad s, 2, R(CH_3)-C=CH_2).

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 77.93; H, 10.20.

Component 7 (retention time 23.8 min), 9.2% yield, was identified as 4-methyl-3-cyclohexenyl methyl ketone (**12**) and had the following characteristics: ir 1705 (C=O), 1688 (C=C),

(22) E. Kuglar and E. Kováts, *Helv. Chim. Acta*, **45**, 1480 (1963).

(23) K. Alder, H. Offermanns, and E. Ruden, *Chem. Ber., B*, **74**, 905 (1941).

1373 and 1347 (two CH_3), 907 cm^{-1} (C=CH); nmr 1.59 (s, 3, $CH_3(R)C=CHR'$), 1.8–2.7 (broad envelope, 7, three CH_2 and $CH_3COCHRR'$), 2.02 (s, 3, $CH_3C=O$), 5.26 (broad s, 1, $CH_3(R)C=CHR'$).

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.02; H, 10.28.

Grignard Addition to Ketones to Form Tertiary Alcohols.—Methylmagnesium iodide was prepared in the usual way by adding 6.1 g (0.04 mol) of CH_3I in ether to 1.0 g (0.04 g-atom) of Mg. To this excess of CH_3MgI, 2 g (0.014 mol) of a mixture of *cis*- and *trans*-2-methyl-2-vinylcyclobutyl methyl ketone (**5** and **6**, respectively) (component 2) in ether was added dropwise. The mixture was gently refluxed for 1 hr and cooled, and the addition compound was decomposed with aqueous NH_4Cl. Analysis by glpc on column C showed only two peaks, which indicated quantitative yields of *cis*- and *trans*-α,α,2-trimethyl-2-vinylcyclobutanemethanol. The quantity of each isomer was about the same; thus, the yield was 1.2% each of the cis and trans isomers from the photocycloaddition. These two isomers were separated on column F.

The compound assigned the cis configuration (in retrospect) (**13**) showed the following spectral characteristics: ir 3580 (OH), 3080 (C=CH), 1630 (C=C), 1377 ($RR'R''CH_3$), 1362 (geminal methyls), 908 cm^{-1} ($C=CH_2$); nmr 0.94 (s, 3, $RR'R''CCH_3$), 1.12 and 1.23 (two s, 6, $(CH_3)_2C(OH)R$), 1.47 (s, 1, OH), 1.67–2.23, (broad m, 5, two CH_2 and $RR'R''CH$), 5.03 (d, 1, cis terminal vinyl H, J_{cis} = 10.5), 5.29 (s, 1, trans terminal vinyl H, J_{trans} = 18), 6.60 (m, 1, J_{cis} = 10.5, J_{trans} = 18, $RCH=CH_2$).

The compound assigned the trans configuration (in retrospect) (**14**) showed the following spectral characteristics: ir 3620 (OH), 3080 ($C=CH_2$), 1632 (C=C), 1378 ($RR'R''CHC_3$) 1361 (geminal methyls), 908 cm^{-1} ($CH=CH_2$); nmr 1.11 (s, 3, CH_3), 1.21 (s, 3, CH_3), 1.39 (s, 3, CH_3), 1.33 (s, 1, OH), 1.5–2.35 (m, 5, two CH_2 and $RR'R''CH$), 5.00 (m, 2, J_{cis} = 10, J_{trans} = 18, $RCH=CH_2$), 6.12 (m, 1, J_{cis} = 10, J_{trans} = 18, $RCH=CH_2$).

Anal. Calcd for $C_{10}H_{18}O$: C, 77.87; H, 11.76. Found for cis alcohol: C, 77.92; H, 11.92. Found for trans alcohol: C, 77.66; H, 11.72.

Preparation of Cis and Trans Diols (15 and 16).—*cis*- and *trans*-α,α,2-trimethyl-2-vinylcyclobutanemethanol were converted to the respective cis and trans diols by hydroboration of the double bond to form a primary alcohol. The procedure was the same as that reported by Brown and Subba Rao.[7b] The diols were purified by glpc on SE-30 (column E).

The diol assigned the cis configuration (in retrospect) (**15**) was a solid, mp 87–87.5°, and had the following characteristics: ir 3300 (broad, strong OH absorption, hydrogen bonded) and no indicated unsaturation; nmr 1.04 (s, 3, CH_3), 1.10 (s, 3, CH_3), 1.17 (s, 3, CH_3), 1.40–2.10 (m, 5, two CH_2 and $RR'R''CH$), 1.93 (t, J = 7, 2, CH_2CH_2OH), 3.07 (broad s, 2, OH), 3.58 (t, J = 7, CH_2CH_2OH).

The diol assigned the trans configuration (in retrospect) (**16**) did not crystallize and had the following spectral characteristics: ir 3300 (broad, strong OH absorption, hydrogen bonded) and no indicated unsaturation; nmr 1.12 (s, 3, CH_3), 1.18 (s, 3, CH_3), 1.24 (s, 3, CH_3), 1.30–2.40 (broad m, 5, two CH_2 and $RR'R''CH$), 1.67 (t, 2, J = 7, CH_2CH_2OH), 3.57 (partially obscured complex t, 2, CH_2CH_2OH), 4.36 (s, 2, OH).

Anal. Calcd for $C_{10}H_{20}O_2$: C, 69.72; H, 11.70. Found for cis diol: C, 69.85; H, 11.69. Found for trans diol: C, 69.53; H, 11.78.

Dehydration to Form *cis*- and *trans*-2-Isopropenyl-1-methylcyclobutaneethanol (1 and 17).—The separate cis and trans diols (0.014 mol each) were refluxed with an excess of acetic anhydride (0.060 mol) for 3 hr. The reaction was monitored at 0.5-hr intervals by glpc on column B (SE-30). The disappearance of the diol peak was coincidental with the appearance of the diacetate which subsequently was replaced by the monoacetate of the unsaturated primary alcohol. Without purification, the reaction mixture was then reduced by addition to 1.5 g of $LiAlH_4$ in 100 ml of ether and refluxing for 2.5 hr. The *cis*- and *trans*-2-isopropenyl-1-methylcyclobutaneethanols prepared from the cis and trans diols, respectively, were purified by glpc (columns E and F) and analyzed spectroscopically. The spectral characteristics of the natural compound 1 were identical with those of the synthetic compound assigned the cis configuration: ir 3630 (free OH), 3250–3350 (H-bonded OH), 1642 and 885 ($C=CH_2$); nmr 4.88 and 4.71 (s, 1, $RR'C=CH_2$), 3.63 (t, 2, CH_2CH_2OH, J = 7.5), 2.60 (t, 1, methinyl H, J = 8.0), 2.59 (s, 1, OH) (the hydroxyl proton resonance shifted upfield on dilution and disappeared with the addition of trichloroacetyl isocyanate), 1.72 (s, 3, vinyl methyl), 1.22 (s, 3, $RR'R''CCH_3$), and 1.3–2.2 (m, 6); mass spectrum (70 eV) *m/e* (rel intensity) 154 (2), 139 (6), 136 (3), 121 (10), 109 (27), 93 (15), 81 (17), 68 (100), 53 (23), 41 (42).

Anal. Calcd for $C_{10}H_{18}O$: C, 77.86; H, 11.76. Found: C, 77.63; H, 11.70.

The *trans*-2-isopropenyl-1-methylcyclobutaneethanol (**17**) was very similar to the cis isomer in glpc behavior, ir, and mass spectra (*vide supra*). The nmr spectrum was 4.80 and 4.60 (s, 1, $RR'C=CH_2$), 3.63 (t, 2 CH_2CH_2OH, J = 7.5), 2.60 (t, 1, methinyl H, J = 8.0), 2.16 (s, 1, OH), 1.85 (t, 2, CH_2CH_2OH, J = 7.5), 1.65 (s, 3, vinyl CH_3), 1.25–1.75 (m, 4), 0.92 (s, 3, $RR'R''CCH_3$), and was identical with a spectrum supplied by Corey[20] and identified as the trans compound.

Anal. Calcd for $C_{10}H_{18}O$: C, 77.86; H, 11.76. Found: C, 77.68; H, 11.89.

1-Hydroxy-3,3-dimethylcyclohexaneacetic Acid (18).—To 20 g (0.36 mol) of potassium hydroxide in 100 ml of water was added 15.0 g (0.07 mol) of ethyl 1-hydroxy-3,3-dimethylcyclohexaneacetate.[13] The stirred mixture became homogeneous in 3 hr at room temperature. After extraction with ether, the aqueous basic solution was acidified, and the precipitated acid was collected by filtration and dried over phosphorus pentoxide for 16 hr. The dried acid was recrystallized from benzene to give 11.0 g (84.6%), mp 109–110°.

Compound **18** had the following nmr characteristics: ($CDCl_3$) 0.91 (s, 3, CH_3), 1.11 (s, 3, CH_3), 1.20–2.10 (broad m, 8, CH_2 ring), 2.51 (s, 3, CH_2COOH), 7.29 (broad s, 2, COOH and OH).

Anal. Calcd for $C_{10}H_{18}O_3$: C, 64.49; H, 9.74. Found: C, 64.55; H, 9.75.

Dehydration of 1-Hydroxy-3,3-dimethylcyclohexaneacetic Acid.—The hydroxy acid (45.1 g, 0.24 mol) was refluxed 6.5 hr in 200 ml of acetic anhydride. The excess anhydride was removed by distillation *in vacuo*. The residue was hydrolyzed with 300 ml of 20% aqueous potassium hydroxide by stirring for 2 hr at room temperature. The aqueous basic solution was extracted with dichloromethane and acidified. The acidic aqueous solution was extracted with dichloromethane, dried over sodium sulfate, and concentrated to 42.4 g of a mixture of all possible double bond isomers. The *Z* acid, prepared by hydrolysis of the corresponding ester (*vide infra*), mp 102.5–104.0°, had the following nmr characteristics: 0.97 (s, 6, geminal CH_3), 1.2–2.0 (broad m, 4, CH_2), 2.20 (broad t, 2, CH_2 trans to carboxyl group), 2.68 (s, 2, CH_2 cis to carboxyl group), 5.73 (s, 1, C=CH). The *E* acid melted at 91.0–92.5°.

Esterification of the Mixed Unsaturated Acids and Separation of the Isomers.—The mixed unsaturated acids, 39.9 g, were refluxed with 150 ml of 14% w/v BF_3–methanol for 1.5 hr. The cooled mixture was poured into 450 ml of water and shaken. The aqueous mixture was extracted with pentane. After the extract was dried over sodium sulfate and the pentane was removed, a residue of 42.8 g remained. The mixture was distilled to yield 35.4 g of mixed esters (85% exo isomers in 1:1 ratio). Separation of the isomers was achieved in a subsequent distillation through an annular spinning band column. The *Z* ester **19** and the *E* ester **20** boiled within 1° of one another (bp 80° at 2.5 m) but were cleanly separated.

Compound **19** had the following nmr spectrum: 0.92 (s, 6, geminal CH_3), 1.2–1.9 (broad m, 4, two CH_2), 2.12 (t, 2, CH_2 trans to carbomethoxy group), 2.61 (s, 2, CH_2 cis to carbomethoxy group), 3.57 (s, 3, $COOCH_3$), 5.55 (s, 1, C=CH).

Compound **20** had the following nmr spectrum: 0.89 (s, 6, geminal CH_3), 1.2–1.9 (broad m, 4, two CH_2), 1.93 (s, 2, CH_2 trans to carbomethoxy group), 2.73 (t, 2, CH_2 cis to carbomethoxy group), 3.57 (s, 3, $COOCH_3$), 5.42 (s, 1, C=CH).

Anal. Calcd for $C_{11}H_{18}O_2$: C, 72.49; H, 9.95. Found for *Z* ester: C, 72.39; H, 10.02. Found for *E* ester: C, 72.68; H, 9.77.

***Z*-3,3-Dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol (2).**—The methyl ester of the *Z*-unsaturated acid collected from glpc (about 100 mg) was added to 1 g of $LiAlH_4$ in 50 ml of ether. The mixture was refluxed 1.5 hr and cooled, and the excess hydride was decomposed with 10% NaOH. The ether solution was filtered and concentrated, and the alcohol was purified by glpc. The glpc behavior and nmr, ir, and mass spectra of natural compound **2** were identical with those of synthetic *Z*-3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol: ir 3610 cm^{-1} (OH); nmr 0.95 (s, 6, $RR'C(CH_3)_2$), 1.13–1.83 (broad m, 4, RCH_2CH_2R'), 2.00 (s, 2) overshadowing 2.09 (m, 2) suggesting two methylenes adjacent to a double

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

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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).