Plant. Cedrela fissilis Velloso.

Occurrence. São Paulo (Campinas), Minas Gerais.

Source. Horto Florestal, Cantareira, São Paulo.

Previous work. On sister species.[1-4]

Seeds (1·6 kg). The petrol extracts gave 327 g (20·4%) of oil. The defatted material was extracted with $CHCl_3$ and the viscous residue (150 g) treated with petrol. The resulting yellowish crystalline-like precipitate (110 g), m.p. 60–80°, was chromatographed on silica gel columns. The benzene–$CHCl_3$ (9:1) eluates furnished 11·58 g (0·72%) of mexicanolide, m.p. 225–228° (MeOH), mixed m.p., co-chromatography and IR spectra with an authentic sample. The benzene–$CHCl_3$ (1:1) eluates afforded 22·89 g (1·43%) of 3-β-hydroxy-iso-mexicanolide[4] which was separated from contaminants by crystallization in pyridine, m.p. 112–118°, raised to 189–194° after recrystallization in Et_2O, mixed m.p., co-chromatography and IR spectra with an authentic sample.

Acknowledgements—The authors are indebted to Dr. Yone Penteado de Castro Pasztor for supplying the plant material, to Dr. T. C. Rizzini for classification of the species, to Dr. C. W. L. Bevan and Dr. D. A. H. Taylor for a sample of mexicanolide. We express our thanks to the *Conselho Nacional de Pesquisas* for financial support and to the *Fundo de Pesquisas do Instituto Butantan* for a research grant. The technical assistance of Mrs. Kazuko Kajibata Gaeta and of Messrs. Sebastião Ribeiro, Teodomiro Vieira Santos and Alípio Raul da Silva is appreciated.

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ONAGRACEAE

TRITERPENES IN THE SEED OIL OF EVENING PRIMROSE, *OENOTHERA LAMARCKIANA*

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Abstract—β-Sitosterol and lupeol have been isolated from the seed oil of *Oenothera lamarckiana* Ser. Two other triterpenes present in trace amounts are citrostadienol and cycloartenyl palmitate.

THE SEED oil of evening primrose (*Oenothera lamarckiana* Ser. Onagraceae) is rich in the glycerides of saturated and unsaturated fatty acids[1] and is of promise in the treatment of

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claudicants.[2] Further information was required on the composition of the non-saponifiable material from *Oenothera lamarckiana* Ser. Previous work in 1961 reported the presence of β-sitosterol (I) with minor phytosterols.[3]

The seed oil was saponified and the non-saponifiable matter, isolated by extraction with hexane, amounted to 1·7%. We have confirmed the presence of β-sitosterol as a major constituent (35% of the non-saponifiable part based on weight isolated, but GLC suggests it may be as much as 65%) by isolation of a pure sample by chromatography on silica. The mass spectrum gave a molecular ion at *m/e* 414 and showed fragmentation of the side-chain and ring D consistent with the structure (Fig. 1). IR, NMR and mass spectroscopic measurements gave identical results as those of an authentic sample. This was confirmed by a mixed melting point determination.

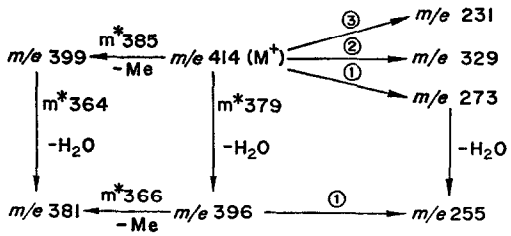

FIG. 1.

In GLC studies of non-saponifiable matter, β-sitosterol has been reported as the major sterol in corn, cottonseed, olive, milo maize, safflower and other oils.[4] Further studies[5] by GLC showed the wide occurrence of a peak which may be attributed to cycloartenol (II) and/or a C_{30} alcohol. Previously Capella[6] isolated cycloartenol from linseed oil and suggested that it might be a common metabolite in vegetable oils.

Triterpenoid fractions A and B were isolated by a combination of column and preparative TLC. No trace of cycloartenol was detected. Although triterpene mixture A showed similar TLC properties to cycloartenol, the absence of a compound containing a cyclopropane ring was evident from the absence of high field signals in the region of τ 9·3–9·8 in the NMR spectrum of the mixture. In addition mass spectral examination also showed the absence of a 9,19-cyclo function seen in cycloartenol and its analogues.[7] On argentation TLC, cycloartenol had a slightly different R_f value to triterpene A. Absence of cycloartenol in *O. lamarckiana* Ser. may be due to its conversion to β-sitosterol.[8] However, cycloartenyl palmitate is present in trace amounts.

The triterpene A was purified via its acetate and proved to be lupeol (III), by NMR, IR and mass spectra of the acetate and free alcohol compared with those of authentic samples, and confirmed by mixed melting point of the acetates.

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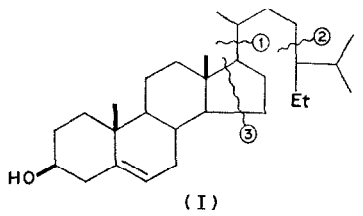
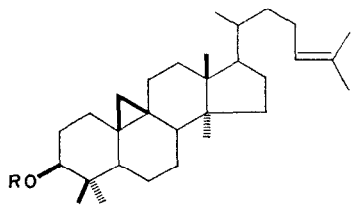
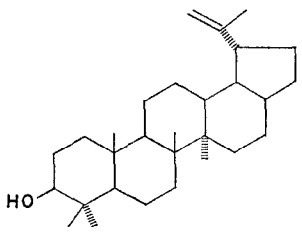


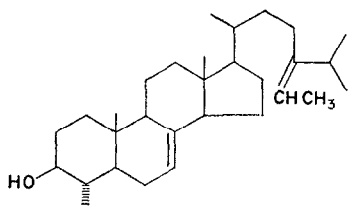
(I)



(II) a ; R = H
b ; R = $CH_3(CH_2)_{14}CO$



(III)



(IV)

The triperpene alcohol B has the formula $C_{30}H_{50}O$ by accurate mass measurement and melts at 165° suggesting citrostadienol (IV)[9] as a possibility and TLC on silica gel corresponds to a possible 4 α-methyl sterol.

The mass spectrum shows a molecular ion at *m/e* 426 which loses 98 m.u. by a McLafferty rearrangement in accord with the properties of a 24-ethylidene group (Fig. 2).[7] The NMR spectrum shows two methyl groups as singlets at 9·46τ and 8·96τ and a doublet ($J = 5$ Hz) at 9·12τ. There also appears to be a broad doublet at 8·5 ($J = 5$ Hz) consistent with the methyl group of a 24-ethylidene group of citrostadienol (IV). Two olefinic protons are present; one at 5·25τ and the other at 4·75τ as broad bands. While the data is in accord with the structure for citrostadienol (IV), shortage of material and lack of an authentic specimen prevented absolute identification.*

A small amount (1 mg) of a palmitic ester of a triterpene was isolated by preparative

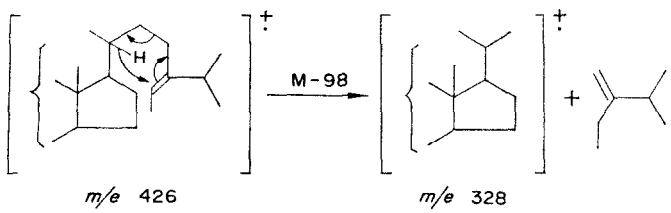


FIG. 2.

* Dr. L. J. Goad (University of Liverpool) has kindly examined our sample of citrostadienol using gas chromatography–mass spectrometry and has confirmed that the major component in our sample is citrostadienol by direct comparison with an authentic specimen. A lower homologue of citrostadienol is also present which is probably 24-methylenelophenol since the molecular ion at *m/e* 412 loses 84 m.u to give a peak at *m/e* 328 (cf. Fig. 2) and thereafter the mass spectrum resembles that of citrostadienol.

[9] Y. MAZUR, A. WEIZMANN and F. SONDHEIMER, *J. Am. Chem. Soc.* **80**, 1007 (1958); **80**, 6293 (1958).

TLC. The IR spectrum of the ester showed a definite shoulder at 3050 cm^{-1} indicative of a cyclopropane ring. The ester function is indicated by C=O at 1730 cm^{-1} and C—O—C at 1260 cm^{-1}. The mass spectral fragmentation is summarized by Fig. 3, and suggests that the palmitic ester is cycloartenyl palmitate (IIb).

Thus while the IR and mass spectral data are in accord with the cycloartenyl palmitate (IIb) lack of material prevented us from isolating the triterpene alcohol by the procedure of Knapp.*,[10]

TLC comparison of the non-saponifiable matter with β-amyrin, parkeol, lanosterol, and stigmasterol failed to detect any of these substances in *Oenothera lamarckiana* Ser.

EXPERIMENTAL

Saponification

The oil (980 g) was saponified at 100° with a KOH (350 g) in EtOH (1·25 l.) and H_2O (0·25 l.) for 1 hr. The mixture was diluted with H_2O (10 l.) and MeOH (10 l.) and extracted with 3 × 5 l. hexane. The combined extracts were concentrated to 5 l. and washed with 3 × 3 l. H_2O 3 × 3 l. 0·5 N KOH, each being followed by 3 l. H_2O. Finally the hexane solution was washed further 4 × 3 l. H_2O till free of alkali. Evaporation after drying ($MgSO_4$) gave the non-saponifiable matter as a pale yellow waxy solid (12·0 g).

Chromatography

998 mg of non-saponifiable matter was separated on a 2 × 30 cm column Kieselgel 0·05–0·2 mm (Merck). Eluting with hexane and increasing Et_2O (1–50%) and then EtOH (0·5–1%) gave 20 fractions. Fractions 1–8 contained largely hydrocarbons (10 mg) and on TLC on silica using 1% Et_2O in hexane gave the palmitic ester of a triterpene (1 mg R_f = 0·2).

Fraction 9 (96 mg) contained ethyl esters of the fatty acids (15 mg) arising from transesterification.

Fraction 12 (72 mg) was subjected to preparative TLC on silica using 2% acetone in benzene for the first elution then 5% acetone in benzene for the second elution. Two triterpene fractions *A* (13 mg) (R_f = 0·25) and *B* (10 mg) (R_f = 0·20) were isolated.

Fraction 13 (525 mg) treated similarly gave more of *A* (1 mg), *B* (13 mg) and β-sitosterol (200 mg), m.p. 134° (lit.[11] 136°) (from MeOH) undepressed on admixture with authentic sample. IR ν_{OH}^{KBr} 3400 cm^{-1}. NMR (τ values in CCl_4) 9·3 (s, 3H, Me) 9·0 (s, 3H, Me), 7·9 (bs, 1H, —OH), 4·7 (bs, 1H, olefinic proton), 7·7–9·3 (complex). The signal at τ 7·9 disappeared on the addition of deuterium oxide. Mass spectrum *m/e* 441 M^+.

Fraction 14 (140 mg) was pure β-sitosterol. Fractions 15 and 16 (22 mg) was β-sitosterol contaminated with yellow pigments. Fractions 17–20 (19 mg) were yellow pigments which were not examined any further.

Triterpene Fraction A

Further purification was achieved by recrystallization from hexane. A low melting wax deposited (150 mg, m.p. 78°) and evaporation of the mother liquors gave a mixture of possibly two triterpenes (50 mg) and these were converted to their acetates with pyridine–Ac_2O at room temp. overnight. A pure sample of the major acetate (25 mg) was obtained by fractional crystallization from MeOH and proved to be lupeol acetate, m.p. 206° (lit.[12] 217°) undepressed on admixture with an authentic sample, m.p. 208°. IR $\nu_{C=O}^{KBr}$ 1733 cm^{-1}. NMR (τ values $CDCl_3$) 9·2 (s, 3H, Me), 9·15 (s, 9H, 3 Me), 9·05 (s, 3H, Me), 8·95 (s, 3H, Me), 8·28 (bs, 3H, Me—C=), 7·95 (s, 3H, MeC O·O), 5·4 (m, 1H, Methine deshielded by oxygen) overlaid with 5·25 and 5·35 (bs, 1H each, olefinic protons). Mass spectrum *m/e* 468 M^+.

Triterpene Fraction B

This had the following characteristics: m.p. 165° (MeOH) lit.[9] m.p. for citrostadienol, 162–164°. IR ν_{OH}^{KBr} 3450 cm^{-1}. NMR. (τ values $CDCl_3$) 9·46 (s, Me), 9·12 (d, Me-CH), 8·96 (s, 3H, Me), (8·5d Me—CH), 4·75 (b, 1H, olefinic), 5·25 (b, 1H, olefinic). Mass spectrum *m/e* (%) 426·3864 M^+ (2·5) ($C_{30}H_{50}O$ requires 426·3862), 411 (4), 328 (10), 285 (20), 269 (3), 111 (40), 97 (74), 83 (87), 69 (84), 55 (100), 43 (63), 41 (50)

* We have also been able to confirm that our sample of cycloartenyl palmitate is identical with authentic material kindly provided by Dr. F. F. Knapp. (See Ref. 10.)

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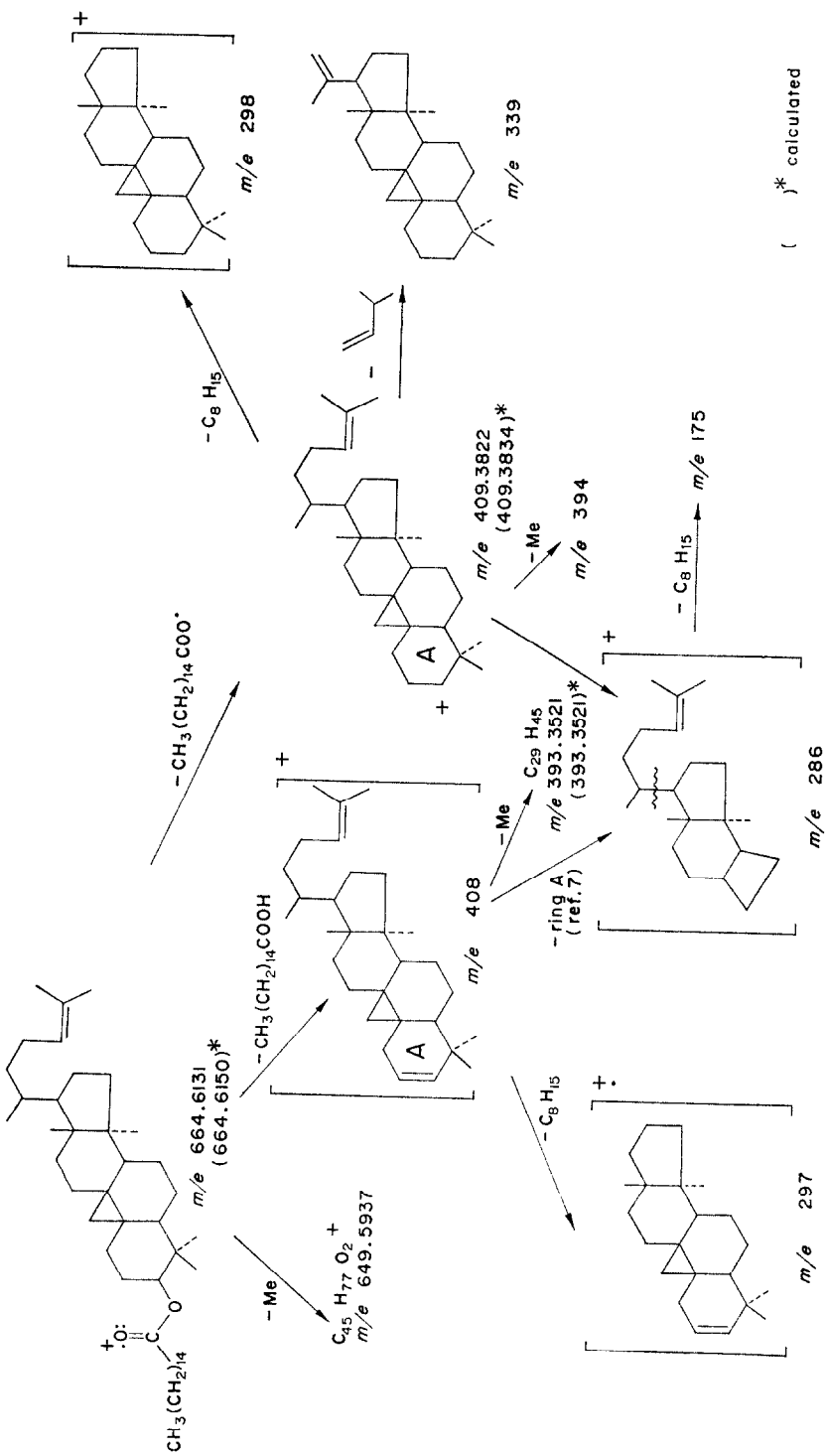

FIG. 3. FRAGMENTATION OF CYCLOARTENYL PALMITATE

Palmitic Ester of a Triterpene

IR (Nujol) 3050 (cyclopropane CH_2) 1730 (C=O) 1260 cm^{-1} (C—O—C). Mass spectrum *m/e* (%) 664·6131 (15) M^+ ($C_{46}H_{80}O_2$ requires 664·6150), 649 (8), 409 (30), 408 (17), 394 (8), 393 (25), 339 (5), 299 (4), 298 (4·5), 297 (5), 286 (4), 271 (6), 257 (7), 239 (10·5), 229 (13), 218 (25), 205 (35), 204 (40), 189 (60), 191 (25), 190 (25), 177 (20), 175 (25), 173 (15), 163 (17), 161 (25), 159 (15), 149 (33), 147 (35), 137 (40), 136 (30); other strong peaks at 135 (55), 123 (50), 121 (57), 109 (88), 107 (60) with base peaks at *m/e* 41, 43, 55 and 57.

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RANUNCULACEAE

IDENTIFICATION OF DELSOLINE FROM *DELPHINIUM AJACIS**

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Plant. Delphinium ajacis—Commonly called Larkspur.

Source. Seeds purchased from G. J. Ball, Incorporated, West Chicago, Illinois, and grown both in the Horticulture Greenhouses and in the field plots of the Agricultural Experiment Station at Stillwater.

Uses. Possess insecticidal properties and plants were responsible for poisoning of cattle in western United States.[1]

Previous work. In earlier communications[2,3] on biosynthesis studies of diterpenoid alkaloids, the isolation of an unknown alkaloid designated as LBA-III was reported. This communication presents the conclusive identification of LBA-III as delsoline (I).[4]

* Journal Article 2135 of the Agricultural Experiment Station, Oklahoma State University, Stillwaterl Oklahoma. This research was supported in part by Grants GB-13126 and GB-20296 from the Nationa, Science Foundation, Washington, D.C.

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