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SESQUITERPENES FROM *FLOURENSIA CERNUA*

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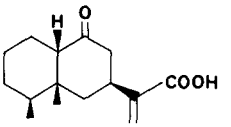
Abstract—The common western shrub *Flourensia cernua* has yielded the eremophilane sesquiterpene flourensic acid and a new aromadendrane sesquiterpene flourensadiol. The toxic component(s) of the plant were found in a petrol-soluble fraction.

INTRODUCTION

The common western shrub *Flourensia cernua* DC has been implicated as the causative agent in various outbreaks of stock poisoning [1]. However, chemical studies on the constituents of the shrub have been conspicuously lacking. Our initial studies revealed the presence of the two flavonoids, hispidulin and cirsimaritin [2], and further studies resulted in the isolation of two new sesquiterpenes which we have named flourensic acid and flourensadiol, together with some miscellaneous acids and esters of known structure. This paper reports the isolation and structures of these materials; a preliminary account of our work on flourensic acid has appeared [3].

RESULTS AND DISCUSSION

Plant material was collected near Big Bend, Texas, in March 1968, and consisted of plant heads only. Extraction of this material with ethanol followed by chromatographic separation of the extract on silica gel or alumina columns yielded several fractions which could be induced to crystallize on standing in appropriate solvents.

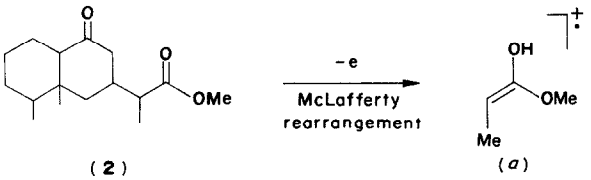


(1)

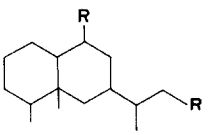
The first compound investigated was named flourensic acid (**1**) on the basis of its acidic properties. It gave analytical data consistent with the composition $C_{15}H_{22}O_3$, and its NMR spectrum showed the presence of both secondary and tertiary methyl groups and also a terminal methylene group. Its UV spectrum (λ_{max} 203 nm; ϵ 8450) supported its formulation as an $\alpha\beta$-unsaturated acid.

Hydrogenation of flourensic acid (**1**) proceeded with the uptake of 1 mol of hydrogen to give a carboxylic acid, dihydroflourensic acid, which yielded a methyl ester (**2**) on methylation with diazomethane. The IR spectrum of this ester indicated the presence of an unstrained ketone (ν_{max}

1706 cm^{-1}) in addition to the ester carbonyl group (ν_{max} 1736 cm^{-1}). It was thus possible to formulate flourensic acid as a bicyclic sesquiterpene with, most probably, a carbonyl group in a six-membered ring. This conclusion was corroborated by the dehydrogenation of a derivative

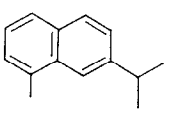


(2) (*a*)



(3) R = OH
(4) R = OTs
(5) R = H

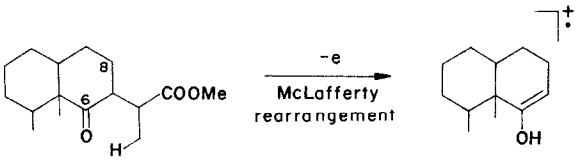
of flourensic acid to eudalene. Dihydroflourensic acid was reduced with lithium aluminium hydride to the diol (**3**) which was converted to the ditosylate (**4**) with *p*-toluene sulfonyl chloride in pyridine, and then to the hydrocarbon (**5**) with lithium aluminium hydride. Treatment of (**5**) with



(6)

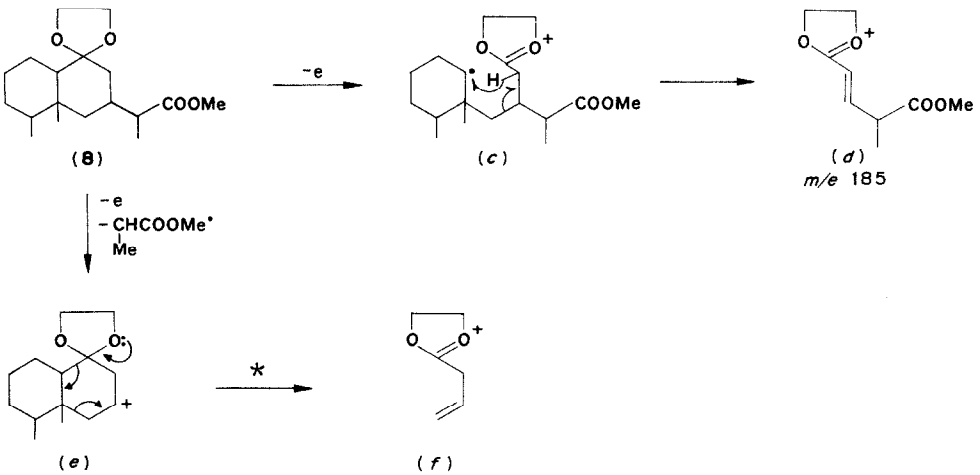
selenium at 340° yielded eudalene (**6**) as the major component of the crude dehydrogenation mixture.

The position of the carbonyl group was established by examination of the MS of the methyl ester of dihydroflourensic acid (**2**) which showed the presence of a peak of moderate intensity at *m/e* 88 corresponding to the ion (*a*) formed by the McLafferty rearrangement [4]. This finding also corroborated the conclusions about the nature of the side chain made on the basis of the dehydrogenation evidence. Significantly, however, there was no ion *b* detectable at *m/e* 180 (after correction for the ^{13}C isotope of the large ion of *m/e* 179 due to cleavage of the side-chain). Since an ion of *m/e* 180 would be expected from a compound such as **7** containing a carbonyl



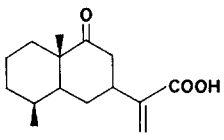
(7) (*b*) *m/e* 180

group in the C-6 or C-8 position, this evidence effectively excludes these positions as possible locations of the carbonyl group. The location of the carbonyl group was finally confirmed by conversion of compound (**2**) to its ethylene ketal (**8**), followed by examination of the MS of this compound. The prominent ion (*d*) at *m/e* 185 can be rationalized by the pathway **8** → (*c*) → (*d*), which is analogous to the fragmentation observed for various steroidal ketals [5, 6]. In confirmation of this formulation of the ion (*d*), an exact mass measurement indicated it had the composition $C_9H_{13}O_4$. A second important peak at *m/e* 113,

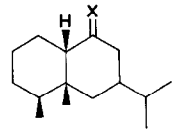


which might have indicated the presence of an α-methyl ketal grouping in (**8**) [5], was found to arise from the cleavage ion (*e*), and is thus formulated as (*f*). This observation serves as a reminder that the ion at *m/e* 113 cannot alone serve as proof of the presence of the α-methyl ketal functionality.

These results indicated that flourensic acid must have the structure (**1**) or (**9**), exclusive of stereochemistry. A choice between these structures was made on the basis of the conversion of the ketal (**8**) to the ketone (**10**) by treatment with lithium aluminium hydride, tosylation with toluene sulfonyl chloride in pyridine, followed by further treatment with lithium aluminium hydride and hydrolysis. Treatment of compound (**10**) with D_2O in base yielded a ketone which had incorporated 3 atoms of deuterium, thus demanding structure (**10**) for the ketone and hence structure (**1**) for flourensic acid.



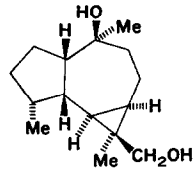
(**9**)



(**10**) X = O
(**11**) X = $NNHC_6H_3N_2O_4$

The structure of the ketone (**10**) was confirmed by conversion to its 2,4-dinitrophenylhydrazone (**11**), identical in all respects (mp, mmp, IR) with the 2,4-DNP derivative of *cis*-tetrahydroeremophilone [7]. The ketone (**7**) had an ORD curve identical to that reported for *cis*-tetrahydroeremophilone thus confirming that its absolute stereochemistry is as indicated. Flourensic acid thus has the absolute stereochemistry shown in (**1**); the possibility of epimerization during the conversion of (**1**) to (**10**) was excluded by the finding that the ORD curve of flourensic acid was very similar to that of *cis*-tetrahydroeremophilone (**10**). Flourensic acid thus belongs to the relatively small class of eremophilane sesquiterpenes [8] and is in fact the first carboxylic acid found with the eremophilane structure and stereochemistry. However, a carboxylic acid with the stereoisomeric valencane skeleton has been reported [9].

The second pure compound obtained was a neutral compound of composition $C_{15}H_{26}O_2$, to which we assigned the name flourensadiol (**12**). Its NMR spectrum showed the presence of two quaternary methyl groups and a secondary methyl group, together with a hydroxymethyl group substituted on a quaternary carbon. The presence of the remaining oxygen function in a tertiary hydroxyl group was inferred from the absence of any chemical shifts in the NMR spectrum that could be attributed to protons on carbon atoms adjacent to an oxygen atom and from the ready loss of water from the (undetectable) parent ion in the MS of (**12**).



(**12**)

Chemical modifications of flourensadiol proceeded only in poor yield to give mixtures of products, and since it formed beautiful crystals suitable for crystallographic analysis, we elected to determine its structure by X-ray crystallography. This work [10] led to the assignment of the structure and stereochemistry represented by (**12**) to this compound. It is of interest to note that flourensadiol gave no colour reaction with tetranitromethane, thus indicating that this test is negative with cyclopropyl carbinols, just as it is with allylic alcohols. Flourensadiol thus belongs to the rare class of aromadendrane sesquiterpenes [8] having the same relative stereochemistry as D-viridiflorol [11]. It is of some interest that the same plant should yield two unusual sesquiterpenes, and suggests that related species should be investigated to determine whether this is a general phenomenon in this genus.

In addition to the two compounds just discussed, a compound identified as (1,3)-arachidobehenicin (**13**) was isolated. Identification was made on the basis of spectroscopic evidence.

$CH_2OCO(CH_2)_{18}Me$
|
CHOH
|
$CH_2OCO(CH_2)_{20}Me$

(**13**)

Because of the reported toxicity of *F. cernua* to sheep and goats [1] we tested our various extracts for toxicity. In view of the difficulties

associated with the use of sheep or goats as assay species, we used the simple and inexpensive goldfish assay [12]. The greatest toxicity was found in the petrol-soluble portion of the crude plant extract, but even in this fraction the toxicity against goldfish was not very high, and we conclude that an assay system more closely modelled on the mammalian system will be required to define the toxicity of this particular plant species.

EXPERIMENTAL

Mps taken in capillaries are uncorr. Elemental analyses were performed by Instranal Laboratory Inc. IR spectra were in CCl_4 unless otherwise stated and UV spectra were in EtOH. The NMR spectra were measured in $CDCl_3$ with TMS as internal standard. GLC was on 10% silicone gum rubber (UCW-98) on Chromosorb W (80–100 mesh). Exact mass measurements by MS were performed at a resolving power of 10000 by the peak matching technique using an appropriate peak of heptacosafluorotri-*n*-butylamine as a reference.

Isolation of flourensic acid (**1**). Whole plant material (158 g flowering heads and some stems) was extracted in a Soxhlet extractor with petrol (bp 60–80°). After evaporation of solvent the residual oil (20·4 g) was dissolved in EtOH and left at 0° for 3 days. Resulting waxes were removed by centrifugation, and the clear supernatant (11·3 g) combined with similar material from earlier extractions. The combined EtOH-soluble material (365 g) was treated at 100°, 1 mm Hg, to remove volatiles, and a portion (175 g) of the involatile residue (298 g) dissolved in CCl_4 (1 litre) and treated with petrol (1 litre). Soluble material (119 g) was separated from a resinuous precipitate.

Chromatography of the CCl_4-soluble material on a silica gel column (7 × 85 cm) eluted with a $CHCl_3$–MeOH gradient gave a total of 127 50 ml fractions. Fractions 66–72, eluted with $CHCl_3$–5% MeOH, were combined on the basis of their weight and TLC behaviour and evaporated to yield crystals of flourensic acid in an oily mother liquor. Recrystallization from Et_2O–$CHCl_3$ yielded pure flourensic acid (2·2 g), mp 160–161°. Fractions 35–40 were also combined on the basis of weight and TLC behaviour. Evaporation and crystallisation from $CHCl_3$ yielded a mixture of arachidic and behenic acids, identified by GLC and MS.

Isolation of flourensadiol (**12**) *and* (1,3)-*arachidobehenicin* (**13**). Plant material (4·2 kg) was extracted in a Soxhlet extractor with EtOH. Evaporation yielded 552 g of crude extract, which was partitioned in the usual way into $NaHCO_3$ soluble, Na_2CO_3 soluble, NaOH soluble, and neutral fractions. The neutral fraction (113 g) was chromatographed on a column of alumina (Baker grade, 850 g). Elution with petrol followed by Et_2O and MeOH gave three fractions. The Et_2O fraction (42 g) was rechromatographed on a similar column. Elution with petrol–Et_2O (1:1) gave a fraction which was crystallized from cyclohexane to yield (1,3)-arachidobehenicin (**13**, 200 mg). Elution with $CHCl_3$ gave a yellow oil in fractions 38–42 which deposited crystals on standing in Et_2O–$CHCl_3$ (20:1). Recrystallization from the same solvent gave pure flourensadiol (**12**, 1·5 g) as colourless crystals, mp 125–126°.

Flourensic acid (**1**). The crystalline material had the following physical properties $[\alpha]_D^{25}$ +60·4° (MeOH; *c* 1·1); ORD M_{376} +226°, M_{336} 0°, M_{311} −779°, M_{303} 0°, M_{260} 4250° (inf), M_{225} +7740°, M_{210} +5010° (last reading). MS: *m/e* (relative abundance), 250 (M^+, 22), 235 (8), 232 (26), 181 (100), 177 (4), 163 (15), 138 (4), 135 (4), 125 (3), 123 (4), 121 (3). UV: λ_{max} 203 nm, ϵ 8450. IR: ν_{max} 3500–3000, (broad), 1705, 1630, 1425, 1088, 955 cm^{-1}. NMR: δ 0·84 (3H, *d*, *J* 6 Hz), 1·04 (3H, *s*), 1·5 (7H, complex), 2·0–2·6 (5H, complex), 5·68 (1H, *s*), 6·42 (1H, *s*). Found: C, 71·93, H, 8·87. $C_{15}H_{22}O_3$ requires: C, 72·0, H, 8·8%.

Dihydroflourensic acid. Flourensic acid (160 mg) was dissolved in EtOH (5 ml) and treated with 10% palladium on charcoal catalyst (50 mg). Hydrogenation was conducted at room temp. and atm pres. and was complete in 2 hr with an uptake of 13·5 ml (corrected to STP; theory for one equivalent is 14·3 ml). The soln was filtered and evaporated to yield dihydroflourensic acid as a colourless gum which did not crystallize. IR: ν_{max} 3500–3000, 1700, 1430, 1380, 1080, 960, 910 cm^{-1}. NMR: δ 0·84 (3H, *d*, *J* 6 Hz), 1·04 (3H, *s*), 1·18 (3H, 2 overlapping *d**), 1·4 (complex), 2·1 (complex). MS: *m/e* (relative abundance), 252 (M^+, 14), 237 (5), 234 (3), 183 (100), 179 (22), 165 (22), 161 (17), 137 (11), 179 (6), 117 (6).

Methyl dihydroflourensate (**2**). Dihydroflourensic acid (100 mg) was treated with a slight excess of ethereal CH_2N_2 and the soln evaporated to yield the ester as an oil which did not crystallise. GLC revealed the presence of two unresolved components in the mixture*. IR: ν_{max} 1736, 1706, 1440, 1375, 1355, 1315, 1230, 1191, 1159, 1090, 1060, 920, 900 cm^{-1}. NMR: δ 0·79 (3H, *d*, *J* 6 Hz), 1·02 (3H, *s*), 1·18 (3H, two overlapping *d*), 1·4 (complex), 2·1 (complex), 3·62 (3H, *s*). MS: *m/e* (relative abundance), 266 (M^+, 15), 251 (5), 248 (3), 235 (5), 207 (5), 197 (100), 179 (50), 165 (20), 161 (20), 137 (13), 123 (6), 121 (5), 109 (22), 107 (6), 105 (8), 95 (11), 88 (58). (Found, *m/e* 266·1884; $C_{16}H_{26}O_3$ requires 266·1881).

Dehydrogenation of flourensic acid. Dihydroflourensic acid (1·96 g) was dissolved in Et_2O and added dropwise under argon to a stirred suspension of 3·2 g $LiAlH_4$ in Et_2O. After 28 hr at room temp. the reaction was quenched with EtOAc and H_2O, and the Et_2O layer separated, dried and evaporated to give 1·50 g of a white solid (**3**) which showed no carbonyl absorption in its IR spectrum. The diol (**3**) (0·32 g) was converted to its ditosylate (**4**) by treatment with *p*-toluene sulfonyl chloride (1·03 g) in dry C_5H_5N–Et_2O (1:10, 50 ml). After 15 hr at 0° the reaction mixture was poured into H_2O at 0°, and the mixture extracted with Et_2O. Usual work-up gave a compound formulated as the ditosylate **4** (0·46 g), based upon the ratio of aromatic to aliphatic protons in its NMR spectrum. A soln of (**4**) in dry Et_2O was added dropwise to a stirred suspension of $LiAlH_4$ (0·038 g) in dry Et_2O in a N_2 atmos. The mixture was refluxed for 4 hr, treated with 0·05 g $LiAlH_4$, and refluxed for a further 14 hr. Usual work-up gave a product (0·18 g) which showed only alkane absorption in its NMR spectrum, and was formulated as **5**. Dehydrogenation of **5** was achieved by treatment with selenium (0·32 g) in a sealed tube at 340° for 14 hr. The crude product was purified by chromatography on alumina and elution with petrol, followed by preparative GLC on a 10 ft × 1/8 in. 10% Apiezon L column at 215°. The major peak eluting at the same time as authentic eudalene (**6**) was collected. It had a UV spectrum showing λ_{max} ($CHCl_3$) 238, 284, 322 nm. Treatment with picric acid in MeOH yielded a picrate, mp 96·5–97·5° (lit [13] 95°). The mp of an authentic sample of eudalene picrate was undepressed in admixture with the above picrate.

* Presumably these observations are caused by the fact that hydrogenation of the double bond creates a new asymmetric centre, and thus both dihydroflourensic acid and methyldihydroflourensate are in fact mixtures of diastereomers.

The MS was similar to that of an authentic sample of **6**.

Methyldihydroflourensate ethyleneketal (**8**). Methyl dihydroflourensate (65 mg) was dissolved in toluene (10 ml) and treated with ethylene glycol (0·5 ml) and *p*-toluenesulfonic acid (50 mg). The flask was fitted with a Soxhlet condenser containing dry $MgSO_4$ in the thimble. The mixture was heated under reflux for 24 hr and GLC showed almost complete conversion of starting material. The reation mixture was cooled, treated with 0·2 ml C_5H_5N followed by H_2O, and the toluene layer was washed, dried and evaporated. The residue was purified by preparative-GLC (1/4 in. SE-30, 225°) to yield material homogeneous on GLC. MS: *m/e* (relative abundance), 310 (M^+, 7), 295 (0·5), 279 (5), 223 (100), 197 (8), 185 (10), 181 (5), 179 (6), 161 (5), 113 (35), 111 (12), 109 (10), 99 (6), 88 (5), 87 (11). (Found: 310·2151; $C_{18}H_{30}O_4$ requires 310·2144. Found: 185·0799; $C_9H_{13}O_4$ requires 185·0814.) The IR spectrum of (**8**) showed no absorption at 1705 cm^{-1}, indicating complete conversion of the ketone to the ketal group.

cis-*Tetrahydroeremophilone* (**10**) *from flourensic acid.* The ketal (**8**) (0·50 g) was dissolved in dry THF (10 ml) and treated with $LiAlH_4$ (0·60 g) at reflux temp for 24 hr. Usual work-up gave a product, 95% pure by GLC, which showed a strong –OH absorption and no carbonyl absorption in its IR spectrum and a molecular ion at *m/e* 282. Treatment of this alcohol (0·50 g) in Et_2O with 1 molar equivalent of *n*-butyllithium at −50°, followed by 1·1 molar equivalents of methane sulfonyl chloride, yielded a crude product showing no hydroxyl absorption in its IR spectrum. This material was treated with 0·50 g $LiAlH_4$ in dry THF for 30 hr at reflux temp. The mixture was worked up in the usual way, and the crude product treated with MeOH and 10% HCl (1:1) for 1 hr at reflux temp. The crude product consisted of one major component, as shown by GLC. A small quantity of this component was isolated by preparative-GLC to give *cis*-tetrahydroeremophilone (**10**). MS: M^+ at *m/e* 222. IR: ν_{max} 2960, 2880, 1709, 1470, 1450, 1425, 1385, 1372, 1320, 1238, 1195, 1170, 1125, 1089, 1035 and 915 cm^{-1}. Treatment of the ketone (**10**) with 2,4-dinitrophenylhydrazine in the usual way yielded a 2,4-dinitrophenylhydrazone (**11**) mp 179–181°.

cis-*Tetrahydroeremophilone from eremophilone.* A sample of eremophilone (0·20 g) was converted to *cis*-tetrahydroeremophilone as previously described [7, 14, 15]. Formation of its 2,4-dinitrophenylhydrazone in the usual way gave authentic (**11**) mp 178–179°, undepressed on admixture with the material prepared from flourensic acid.

Deuterium exchange of the ketone (**10**). The crude ketone (**10**) prepared from flourensic acid, was treated with Na in a D_2O–dioxane mixture [16]. After two such exchanges the product was isolated and purified by preparative GLC. Its molecular ion was observed at *m/e* 225 indicting the exchange of three hydrogen atoms by deuterium.

Flourensadiol (**12**). The compound had the following spectral properties: IR ν_{max} 3620, 3400, 1460, 1380, 1260, 1220, 1200, 1170, 1140, 1120, 1100, 1060, 1015, 990, 940, 890, 870, 850 cm^{-1}. NMR: δ 3·72 (1H, *d*, *J* 12 Hz), 3·58 (1H, *d*, *J* 12 Hz), 2·2–1·4 (*c*. 13H, complex), 1·16 (3H, *s*), 1·15 (3H, *s*), 0·98 (3H, *d*, *J* 6 Hz), 0·70 (1H, *m*), 0·35 (1H, *m*). MS: *m/e* (relative abundance), 220 (58, M-18), 202 (75, M-36), 162 (55), 147 (43), 139 (65), 125 (60) and 107 (62). Found: C 75·20, H 10·56%; $C_{15}H_{26}O_2$ requires C 75·58, H 10·97%.

Reaction of flourensadiol with tetranitromethane. Flourensadiol (10 mg) was dissolved in 0·25 ml $CHCl_3$ and mixed with an equal vol. of tetranitromethane in $CHCl_3$ (1:1). No colour formation was observed when the soln was compared with a blank determination [17].

(1,3)-*Arachidobehenicin* (**13**). The material isolated as described had mp 70·5–71·5°; IR: ν_{max} 3300 and 1750 cm^{-1}; NMR: strong resonance at δ 1·3 and weak multiplets at 0·9, 2·0 and 3·7 ppm; MS: *m/e* (relative abundance), 438 (2), 425 (5), 424 (2), 415 (3), 397 (19), 396 (6), 387 (2), 383 (4), 369 (16), 368 (6), 355 (3), 351 (4), 340 (6), 323 (13), 322 (3), 312 (4), 304 (3), 295 (10), 269 (2), 185 (5), 171 (4), 129 (28), 98 (42), 97 (39), 85 (35), 83 (47), 73 (26), 71 (61), 69 (46), 57 (100). The ions at *m/e* 383 [$(M\text{-}C_{19}H_{39}COOCH_2)^+$ and 355 $(M\text{-}C_{21}H_{43}COOCH_2)^+$] were consistent with the formulation of (**13**) as (1,3)-arachidobehenicin, and the remainder of the MS agreed with this assignment [18].

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