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Non-volatile Constituents of Deertongue Leaf

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Examination of the non-volatile constituents obtained from dried deertongue leaves revealed the presence of β-amyrin and 11-oxo-β-amyrin together with the corresponding palmitates, β-amyrin acetate, α- and β-amyrenone, 11-oxo-α-amyrin, cycloartenyl palmitate, β-sitosterol, stigmasterol, (±)-eudesmin, and (±)-epieudesmin. The 11-oxo-β-amyrin derivatives are apparently new natural products.

Deertongue, *Carphephorus odoratissimus* (J. F. Gmel) Hebert (Compositae) is a plant native to south-eastern USA. Due to its high content of coumarin, the oleorosin from deertongue is frequently used as a fixative in perfumery and the dried leaves as a flavour additive to tobacco.

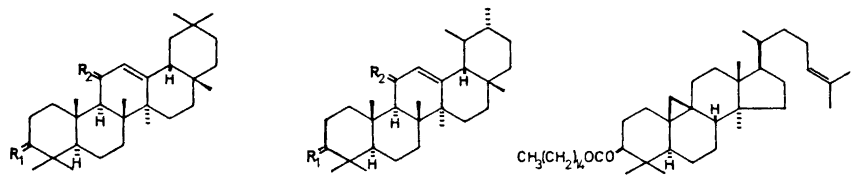
The occurrence in deertongue leaves of lupeol and α-amyrin, the corresponding palmitates and acetates, lupenone, and the volatile components coumarin, dihydrocoumarin, and 2,3-benzofuran has recently been reported.[1] The present investigation of other low-polarity non-volatile components, derived from a hexane-soluble fraction of an acetone extract of dried deertongue leaves, is an extension of that study.

RESULTS

The isolation of individual components was accomplished primarily by repeated chromatography on ordinary and $AgNO_3$-impregnated silica gel. Alcoholic components were acetylated prior to separation on $AgNO_3-SiO_2$.

In addition to the previously reported triterpenoids of lupane and α-amyrin type, deertongue proved to contain further α-amyrin derivatives as well as cycloartenyl palmitate and a series of β-amyrin derivatives. Thus, β-amyrin (*1*), β-amyrin palmitate (*2*), and β-amyrin acetate (*3*) were present in amounts almost equivalent to the corresponding α-amyrin derivatives, whereas cycloartenyl palmitate (*4*) was a minor constituent.

A small fraction containing triterpene ketones, not easily separable by liquid chromatography, consisted of a 1 : 1 mixture of α- and β-amyrenone (*5* and *6*, resp.) according to the NMR results. This tentative assignment was reinforced by GLC studies involving co-chromatography with authentic samples.



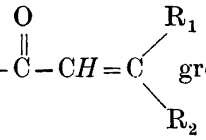
1 R_1 = H,OH(β) R_2 = H_2
2 R_1 = H,OCO$(CH_2)_4CH_3$(β) R_2 = H_2
3 R_1 = H,OCOCH_3 (β) R_2 = H_2
6 R_1 = O R_2 = H_2
8 R_1 = H,OH(β) R_2 = O

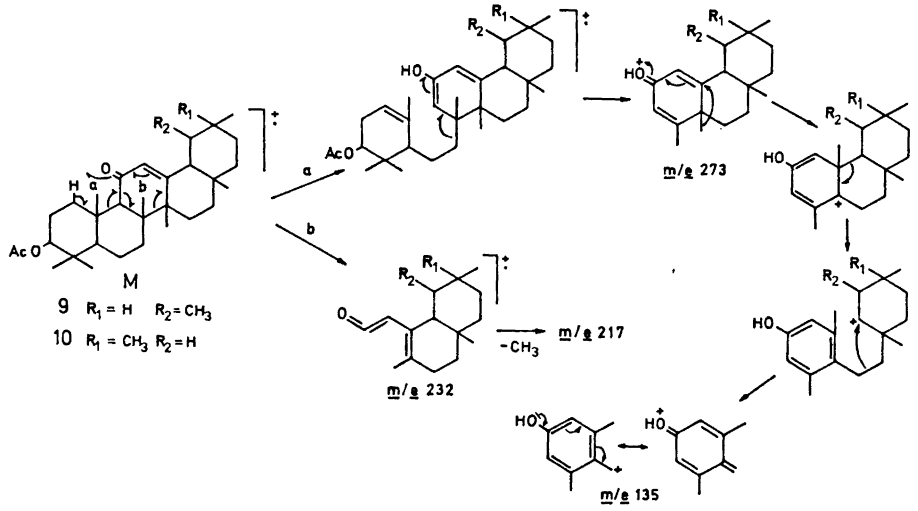
5 R_1 = O R_2 = H_2
7 R_1 = H,OH(β) R_2 = O

4

Fig. 1.

Furthermore, when subjected to GLC-MS analysis the two components gave mass spectra indistinguishable from those of α- and β-amyrenone.

Two triterpene alcohols (*7* and *8*), isolated after conversion to the acetates, had similar spectral properties and each contained an α,β-unsaturated ketone function (ν_{max}: 1665 and 1618 cm^{-1}, λ_{max}: 245 nm, $\log \varepsilon \sim 4.1$). The NMR spectrum displayed a one proton signal at δ 5.62 (δ 5.65, resp.) compatible with the presence of a  group. The mass spectra showed diagnostically significant peaks at *m/e* 482 (M, $C_{32}H_{50}O_3$), 273 ($C_{19}H_{29}O$), and 232 ($C_{16}H_{24}O$) (Scheme 1) indicating that the compounds were 11-oxo-α-amyrin acetate (*9*) and 11-oxo-β-amyrin acetate (*10*).[2] A prominent peak at *m/e* 135,

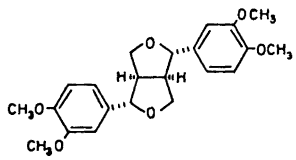


Scheme 1.

essentially due to an ion formed as shown in Scheme 1 and characteristic of 11-oxo-α-amyrin and 11-oxo-β-amyrin derivatives, was also present in the mass spectra.[3] Final proof of the structures of compounds *9* and *10* was afforded by comparison with synthetic samples, which were prepared from α- and β-amyrin acetate by oxidation with chromium trioxide in acetic acid.

A palmitate with molecular weight 678, related to these compounds, was isolated from one of the less polar fractions. The compound could be formulated as 11-oxo-β-amyrin palmitate, since saponification and acetylation of the resulting triterpene alcohol gave an acetate identical with the corresponding synthetic sample. To our knowledge, both 11-oxo-β-amyrin (*8*) and 11-oxo-β-amyrin palmitate are new natural products, whereas 11-oxo-α-amyrin (*7*) has previously been encountered in a few botanical species.[4–6] The β-amyrin derivatives, 3,11-dioxo-olean-12-ene and the corresponding 6β-hydroxy derivative (gymnosporol) have recently been isolated from *Gymnosporia rothiana* Laws.[7]

β-Sitosterol and stigmasterol proved to be the only sterol components. The presence of the latter compound was established by acetylation and bromination of the sterol mixture followed by isolation of stigmasteryl tetrabromo acetate. The identification of β-sitosterol was based mainly on GLC and GLC-MS studies of the acetylated sterol mixture.



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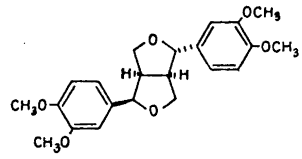

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Fig. 2.

Two compounds, isolated from the most polar fraction, gave spectra (IR, NMR, and MS) in perfect agreement with those of eudesmin (*12*) and epieudesmin (*13*). However, neither possessed optical activity, and the isolated sample of eudesmin had a melting point similar to that reported for (±)-eudesmin.[8] Accordingly, the deertongue components are racemates of eudesmin and epieudesmin. Although lignans are normally optically active,[9] a few examples of naturally occurring compounds lacking optical activity are known, *i.e.* (±)-sesamin, isolated from *Fagara xanthoxyloides* Lam,[10] thomasic acid, thomasidioic acid, and (±)-lyoniresinol from *Ulmus thomasii* Sarg.[11,12]

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 instrument in CCl_4 and NMR spectra on a Varian A-60A spectrometer at 60 MHz in $CDCl_3$ using TMS as internal reference. Rotations were measured on a Perkin-Elmer 141 polarimeter in $CHCl_3$ and UV spectra on a Beckman DK-2A instrument in ethanol.

Analytical GLC was carried out on a Varian 1700 instrument using either a 30 m steel column (i.d. 0.5 mm) coated with Apiezon L, a 2 m steel column (i.d. 3.2 mm) packed with 2 % OV 17 on Chromosorb G or a 2 m steel column (i.d. 3.2 mm) packed

with 1.5 % SE-30 on Chromosorb W. The carrier gas flow was 3 ml N_2/min (capillary column) or 30 – 60 ml N_2/min (packed column). GLC in combination with mass spectrometry was carried out on an LKB 9000 instrument, incorporating a home-made gas chromatograph equipped with both a capillary and a packed column injector, a device for introduction of make-up gas, a splitter and a flame ionisation detector. The columns described above were used also in the GLC-MS studies but nitrogen was substituted for helium as a carrier gas. The temperature of the separator was 260° and that of the ion source 290°. The electron energy used was 70 eV. Mass spectra of nonvolatile compounds were recorded using the direct inlet system. The high-resolution measurements were performed on an Atlas SM 1 and on an MS 902 instrument.

Liquid chromatography was carried out on silica gel (Merck 0.05 – 0.20 mm, activity I and II), on neutral alumina (Schuchardt, activity III) and on $AgNO_3$-impregnated silica gel. Thin-layer chromatography was performed on silica gel Merck GF and on silica gel impregnated with $AgNO_3$ (23 %).

Extraction and separation. Commercially available deertongue leaves (2000 g) were extracted for 24 h with acetone yielding a viscous green gum (224 g, 11 %), which was distilled at reduced pressure (90°, 0.01 mmHg) using CO_2 as a carrier gas.[13] The resulting non-volatile fraction (187 g, 9 %) was dissolved in acetone (200 ml) and subsequently precipitated with hexane (6 l) while stirring. The hexane-soluble material (75 g, 4 %) was fractionated in the usual way into neutrals (62 g, 3 %) and acids (13 g, < 1 %). The neutral material (60 g) was subjected to gradient chromatography on silica gel (hexane-isopropyl ether-ether-ethanol) giving fractions 1 – 8.

Fraction 1 (0.36 g) consisted of a complex mixture of hydrocarbons and was not investigated further.

Fraction 2 (9.46 g). Part (4 g) of this fraction was separated by chromatography on $AgNO_3$-impregnated silica gel (light petroleum-isopropyl ether) into four constituents, identified as α-amyrin palmitate [1] (0.4 g), lupenyl palmitate [1] (1.2 g), β-amyrin palmitate (*2*, 0.4 g), and cycloartenyl palmitate (*4*, 0.09 g).

β-Amyrin palmitate (*2*), after recrystallisation from chloroform-methanol, had m.p. 70 – 73°; $[\alpha]_D$ + 66° (*c* 0.8) (reported m.p. 75 – 76°; $[\alpha]_D$ + 67.9°)[14]; ν_{max}: 1728 and 1175 cm^{-1}; δ: 4.56 (1H, dd) and 5.23 (1H, m); *m/e* (%): 664 (5), 445 (1), 409 (10), 408 (20), 218 (100), 203 (29), and 189 (18). The compound (90 mg) was refluxed with aqueous KOH (0.5 ml, 45 %) in ethanol for 2 h under nitrogen. Dilution with water, acidification and extraction with ether, yielded a mixture which was treated with ethereal diazomethane. Subsequent chromatography on silica gel gave methyl palmitate (29 mg) identified by IR and GLC-MS (Apiezon L column), and β-amyrin (*1*, 43 mg) m.p. and mixed m.p. 196 – 198°; the IR, NMR, and mass spectra were identical to those of an authentic sample.

Crude cycloartenyl palmitate (*4*, 0.09 g) m.w. 664, was saponified in the same way as described for β-amyrin palmitate. A small part of the reaction mixture was methylated using diazomethane. Subsequent analysis by GLC-MS showed the presence of methyl palmitate (Apiezon L column). The remainder of the reaction mixture, after acetylation and chromatography on $AgNO_3$-impregnated silica gel, gave as the main component cycloartenyl acetate, m.p. 118.5 – 120°; mixed m.p. 118 – 121°; $[\alpha]_D$ + 58° (*c* 1.1) (reported + 58°);[15] the IR, NMR, and mass spectra were identical to those of an authentic sample. ν_{max}: 1730, 1245, 1040, 1025 and 978 cm^{-1}; δ: 0.33 (1H, d, *J* = 4), 0.59 (1H, d, *J* = 4), 0.87, 0.90, 0.97 (corresponding to five methyl groups), 1.63 (3H, s broad), 1.70 (3H, s broad), 2.05 (3H, s), 4.55 (1H, dd), 5.18 (1H, t); *m/e* (%): 468 (5), 453 (4), 408 (26), 393 (24), 365 (12), 339 (13), 286 (16), 271 (9), 175 (23), 69 (100).

Fraction 3 (5.38 g) was complex and therefore divided into several simpler fractions by repeated chromatography on ordinary and $AgNO_3$-impregnated silica gel. In this way the presence of α-amyrin acetate [1] (0.06 g), lupenyl acetate [1] (0.25 g), and lupenone [1] (0.06 g) could be confirmed. In addition the following components were encountered:

β-Amyrin acetate (*3*, 0.07 g), which on recrystallisation from isopropyl ether-methanol had m.p. and mixed m.p. 239.5 – 242.5° and was identical in all respects to an authentic specimen.

According to NMR spectroscopy α- and β-amyrenone (*5* and *6*, 0.04 g) were present as a 1 : 1 mixture and were only partially separated by liquid chromatography. Complete separation was accomplished by GLC (OV-17 column). These ketones were also identified by co-chromatography with authentic samples and by GLC-MS giving mass spectra indistinguishable from those of α- and β-amyrenone.

Crude 11-oxo-β-amyrin palmitate (*11* 0.12 g), recognised from its mass spectrum, was saponified, treated with ethereal diazomethane and subsequently chromatographed on silica gel (light petroleum-isopropyl ether) giving a non-polar fraction (33 mg). This consisted of methyl palmitate according to GLC-MS (Apiezon L column). The main polar fraction obtained (35 mg) was acetylated and recrystallised from isopropyl ether to give 11-oxo-β-amyrin acetate (*10*) m.p. and mixed m.p. 260 – 264°. The compound was identical in all respects to a synthetic sample (*vide infra*).

Fraction 4 (5.21 g) was a mixture of waxes and triterpene and sterol esters of long-chain fatty acids. It was not investigated further.

Fraction 5 (8.99 g). Part of this fraction (2 g) was acetylated and subsequently chromatographed on $AgNO_3$-impregnated silica gel to give α-amyrin acetate [1] (0.22 g), lupenyl acetate [1] (1.0 g), and β-amyrin acetate (*3*, 0.14 g), m.p. and mixed m.p. 240 – 242°, identical in all respects to an authentic sample.

Fraction 6 (0.34 g) was small and complex and was not investigated further.

Fraction 7 (4.77 g) was subjected to acetylation and repeated liquid chromatography to give three main fractions containing sterol acetates (0.28 g), triterpene acetates (0.11 g) and coumarin (1.5 g), respectively.

The sterol acetate fraction, on GLC analysis (SE-30 column) was shown to consist of two components, having retention times identical to those of β-sitosterol acetate and stigmasterol acetate. The presence of these acetates was corroborated by GLC-MS giving mass spectra identical to those of authentic samples. Furthermore, the IR spectrum displayed peaks at 960 and 972 cm^{-1}, the relative intensities of which indicated a mixture of β-sitosterol acetate and stigmasterol acetate in about 1 : 1 ratio. Part of the sterol acetate mixture (50 mg) was dissolved in ether (1 ml) and treated with a solution (5 ml) of Br_2 (0.22 ml) and NaOAc (50 mg) in acetic acid for 1 h. Excess Br_2 was reacted with aqueous $NaHSO_3$. Work up and recrystallisation from a mixture of chloroform and methanol gave stigmasteryl tetrabromo acetate, m.p. and mixed m.p. 194 – 200° (decomp.); the IR spectrum was identical to that of an authentic sample.

The mixture of triterpene acetates was recrystallised from hexane and from isopropyl ether to give 11-oxo-α-amyrin acetate (*9*), m.p. and mixed m.p. 286 – 289°; $[\alpha]_D + 94°$ (reported: + 96°),[16] M = 482.3759; $C_{32}H_{50}O_3$ requires 482.3760. The UV, IR, NMR, and mass spectra were identical to those of a synthetic sample (18 mg) prepared from α-amyrin acetate (32 mg) by oxidation with CrO_3 (28 mg) in acetic acid (6 ml, 1/2 h, 20°). λ_{max}: 247 mμ (log ε = 4.09); ν_{max}: 1730, 1665, 1618, 1245 cm^{-1}; δ: 2.06 (3H, s), 2.38 (1H, s), 4.6 (1H, dd), 5.62 (1H, s); *m/e* (%, composition): 482 (9, $C_{30}H_{50}O_3$), 467 (1), 422 (75, $C_{30}H_{46}O$), 407 (47), 379 (33, $C_{27}H_{39}O$), 273 (98, $C_{19}H_{29}O$), 232 (100, $C_{16}H_{24}O$), 217 (23, $C_{15}H_{21}O$), 135 (91, $C_{10}H_{15}$, $C_9H_{11}O$). The mother-liquors on chromatography on silica gel (light petroleum-isopropyl ether) gave 11-oxo-β-amyrin acetate (*10*) which after recrystallisation from methanol had m.p. and mixed m.p. 260 – 263°; $[\alpha]_D + 107°$ (reported: + 102°);[16] M = 482.3765; $C_{32}H_{50}O_3$ requires 482.3760. The spectral data were identical to those of an authentic sample prepared from β-amyrin acetate by oxidation with CrO_3/HOAc. λ_{max}: 248 mμ (log ε = 4.06); ν_{max}: 1730, 1663, 1618, 1245 cm^{-1}; δ: 2.07 (3H, s), 2.42 (1H, s), 4.5 (1H, dd), 5.65 (1H, s); *m/e* (%): 482 (11), 467 (2), 422 (61), 407 (42), 379 (26), 273 (63), 232 (100), 217 (29), 135 (79).

Fraction 8 (12.6 g) was chromatographed on neutral alumina (III, isopropyl ether-ether-ethanol) to give as main components, epieudesmin (*13*, 0.5 g) and eudesmin (*12*, 0.6 g). The former compound on recrystallisation from isopropyl ether had m.p. 125 – 127°, mixed m.p. 127 – 129°, $[\alpha]_D \pm 0°$, and was identical in all other respects to an authentic sample. ν_{max}: 1080 cm^{-1}; *m/e* (%): 386 (61), 355 (6), 219 (3), 177 (60), 166 (60), 165 (100), 151 (74).

Eudesmin (*12*), on recrystallisation from hexane-ethyl acetate, had m.p. 90 – 93, 99 – 100° and mixed m.p. 89 – 92°; $[\alpha]_D \pm 0°$; M = 386.1722; $C_{22}H_{26}O_6$ requires 386.1729. The IR, NMR, and mass spectra were identical to those of an authentic sample; ν_{max}: 1062 cm^{-1}; *m/e* (%, composition): 386 (53), 355 (6), 219 (12, $C_{13}H_{15}O_3$), 177 (46, $C_{11}H_{13}O_2$), 166 (27, $C_9H_{10}O_3$), 165 (100, $C_9H_9O_3$), 151 (42, $C_8H_7O_3$, $C_9H_{11}O_2$). Detailed NMR data for these two compounds have previously been published by Atal *et al.*[17]

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