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PENTACYCLIC TRITERPENOIDS FROM LEAVES OF SOME DIOSPYROS SPECIES

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α-Amyrin and bauerenol were isolated from the leaf extracts of *Diospyros kirkii* Hiern and *D. mespiliformis* Hochst ex A. DC. A mixture of three triterpenoid acids was obtained from each of the above extracts, as well as from leaves of *D. usambarensis* F. White. Betulin and betulinic acid were isolated from leaves of *D. consolatae* Chiov. Betulinic acid and α-amyrin were obtained from leaves of *D. verrucosa* Hiern and *D. cornii* Chiov, respectively, lupeol being obtained from the bark of the latter plant as well. One saturated keto alcohol was isolated from twigs of *D. usambarensis* and another from *D. consolatae* bark. C13-NMR spectra of bauerenol and bauerenyl acetate are partially assigned.

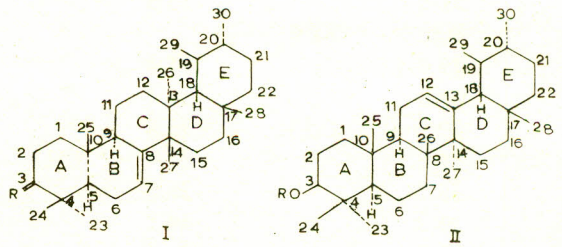
INTRODUCTION

Diospyros is the largest of the genera belonging to the family Ebenaceae [1]. The plants of this genus are widely distributed throughout the tropical and subtropical regions [2] where they are commonly used as folk-medicines [3]. The medicinal potence of the root and stem barks of these plants could be attributed to the naphthoquinones. Some of these compounds have been reported as demonstrating antibacterial and antifungal properties [4, 5] and *Diospyros* species are known to be rich sources of naphthoquinones [6]. As far as we are aware triterpenoids have not been isolated from *D. kirkii* Hiern, *D. mespiliformis* Hochst ex A. DC., *D. cornii* Chiov., *D. usambarensis* F. White, *D. consolatae* Chiov and *D. verrucosa* Hiern, although Fallas, *et al.* [7] report detection of some triterpenoid materials in the stem bark of *D. mespiliformis.*

RESULTS AND DISCUSSION

Fresh or dried leaves of the plants† collected from different parts of Tanzania were investigated for triterpenoids.

A mixture of two triterpenoids was isolated from the neutral fraction of the leaf extract of *D. kirkii.* GLC of the acetylated mixture showed two major (80 and 16% of the mixture) and one minor compound (4%). The GLC–MS of the mixture showed that the major acetate was related to that of bauerenone (Ic) [8]



I

Ia: R=H,OH
Ib: R=-O-CO-CH_3,H
Ic: R=O

II

IIa: R=H, O
IIb: R=CH_3-C-

Chromic acid oxidation of the unacetylated mixture afforded only one (GLC) oxo-compound, the PMR, MS, m.p. and $[\alpha]_D^{25}$ of which were identical with those of bauerenone (Ic) [8, 9]. The MS of the second major acetate (16% of the mixture) was identical with that of α-amyrin acetate (IIb) [8]. The neutral fraction of the leaf extract of *D. mespiliformis* also gave a mixture of α-amyrin and bauerenol.

Triterpenoid acid mixtures were isolated from the acidic fractions of the leaf extracts of *D. kirkii, D. mespiliformis* and *D. usambarensis.* From the comparison of their PMR and MS with those of known compounds [10–13], each of these mixtures appeared to constitute a mono-, a di- and a trihydroxy triterpenoid acid. The mixtures could not be identified on the basis of the available evidence.

Betulin and betulinic acid were isolated for *D. consolatae* leaves. Betulinic acid was also isolated from *D. verrucosa* leaves while α-amyrin was obtained from leaves of *D. cornii,* lupeol being isolated from the bark of the latter plant as well.

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†The plants were authenticated at the herbarium, Botany Department, University of Dar es Salaam, where specimen are deposited.

Table 1. C13-NMR spectral data of bauerenol and bauerenyl acetate.*

Peak No	Bauerenol† Chemical shift	C-atom	Bauerenyl acetate Chemical shift	C-atom
1	145.3	C-8	170.8	*C*=0
2	116.5	C-7	145.3	C-8
3	79.1	C-3	116.2	C-7
4	54.9	C-18	81.0	C-3
5	50.4	C-5	54.8	C-18
6	48.3	C-9	50.5	C-5
7	47.2		48.1	C-9
8	46.8		41.1	C-14
9	41.2	C-14	39.5	C-19/or 20
10	38.0		37.9	C-1
11	37.7	C-4	37.6	C-4
12	35.3		36.4	
13	32.4		35.3	
14	32.0		35.0	
15	31.5		32.3	
16	30.6	31.9		
17	29.7		31.4	
18	29.2		29.1	
19	27.5	C-23	28.8	
20	26.2		27.4	C-23
21	25.7		25.5	
22	24.2		24.1	
23	23.7		23.9	
24	22.5		23.5	
25	21.9		23.3	
26	14.6	C-24	23.1	
27	13.0	C-26	22.5	
28	–		22.4	
29	–		21.2	CH_3
30	–		16.7	
31	–		15.7	C-24
32	–		12.9	C-26

*The spectrum of bauerenol was taken on a JOEL FX60 C13-NMR spectrometer while that of bauerenyl acetate was taken on a Varian CFT -20 instrument. Chemical shift in ppm downfield from internal standard tetramethyl silane, solvent deuterochloroform.

†In the spectrum of the mixture of bauerenol and α-amyrin some peaks might coincide. Therefore, only 27 peaks of bauerenol have been detected.

It appears that in each plant where bauerenol is detected α-amyrin is also found, but not vice versa. This is also observed in other *Diospyros* species [14–17] and in some other plants [9, 18]. This observation is in line with the reported easy isomerization of bauerenol to α-amyrin [19].

A saturated keto-alcohol (molecular formula $C_{54}H_{108}O_2$) was isolated from the twigs of *D. usambarensis* while another one related to it was obtained from *D. consolatae* bark. The structures of these compounds could not be established at this stage.

C13-NMR Spectra of Bauerenol and Bauerenyl Acetate. From the C13-NMR spectra of the mixture of bauerenol (Ia) and α-amyrin (IIa) the signals of each compound could easily be distinguished by comparison with the known chemical shifts of (IIa) [20, 21]. Similarly the signals of their acetates could be distinguished in the spectrum of the acetate mixture. The C13-NMR chemical shifts of (Ia) and its acetate (Ib) are given in Table 1.

Helpful for the assignment of a number of carbon atoms of (Ib) was a comparison of the proton noise decoupled spectrum with off-resonance proton decoupled and gated decoupled spectra, as well as with the reported spectrum of α-amyrin acetate [21]. For (Ia) distinctions were based upon the assignments for (Ib).

From reported C13-NMR spectra of triterpenoids and steroids [20–24] it appears that carbocyclic methine signals occur well on the low field side of the saturated carbon region of the spectra. The lowest field methine signals are given by bridgehead carbons, particularly if flanked by an equatorial ring substituent [20]. From these considerations, therefore, the signals at 54.9 and 54.8 ppm in the spectra of (Ia) and (Ib), respectively, could be assigned to C-18. The signals at 50.4 (Ia) and 50.5 ppm (Ib) could be assigned to C-5. These signals appear at higher field than the comparable ones in α-amyrin and its acetate. This can be explained by the diamagnetic shielding of C-5 by the doulbe bond in (Ia) and (Ib) [22]. The signals at 48.3 (Ia) and 48.1 ppm (Ib) were assigned to C-9. The signal at 39.5 ppm in the spectrum of (Ib) can be attributed either to C-19 or to C-20 or to both carbon atoms.

In the gated decoupled spectrum of (Ib) four singlets were detected at 41.1, 37.6, 35.0, and 31.9 ppm which were assigned to quaternary carbon atoms. On comparison of these signals with those of α-amyrin acetate (IIb) the first signal could be assigned to C-14 and the second one to C-4. The other signals could not be assigned.

The off-resonance spectrum of (Ib) clearly revealed six quartets at 27.4, 23.5, 22.4, 21.2, 15.7 and 12.9 ppm. The first, fourth and fifth signals were assigned to C-23, carbomethoxy methyl carbon and C-24, respectively (compare the spectrum of (IIb) [21]). The highest field signal at 12.9 ppm was attributed to C-26 since this carbon atom is sterically hindered by the *cis*-fused rings D and E. The other methyl and all the methylene carbons except C-1 could not be assigned. Assignments are shown in Table 1.

EXPERIMENTAL

TLC was performed on silica gel (Chromalay, May and Baker) plates with silver nitrate (20% w/w impregnation and benzene – cyclohexane (2:3 v/v) elution [25] which was found to be the best system for triterpenoids.

The leaf extracts were obtained by continuously extracting either dried powdered or shredded fresh leaves in a Soxhlet apparatus for 18 hr with methanol. The extracts were then decolourised with activated charcoal.

Diospyros kirkii *Hiern*

The concentrated crude extract from dried leaves (860 g) of the plant was extracted with ether and then with chloroform. The ethereal fraction was treated with sodium hydroxide solution (10%) to give a neutral (A) and an acidic fraction (B).

Fraction A. It gave white needles (acetone), (2.52 g, 0.29%). The material was found to be a mixture of two compounds, one of them wax α-amyrin (by comparison with an authentic sample). The mother liquor probably contained lupeol (TLC).

GLC–MS of the acetylated mixture showed two acetates (80 and 16% of the mixture, respectively) and a minor unknown compound (4%). The major acetate showed prominent *m/e* values at 468 (M^+), 219, 218 (base peak), 207, 203, 189 and 133. The MS of the second major acetate was identical with that of α-amyrin acetate [8].

Bauerenone. Chromium trioxide-oxidation [14] of the unacetylated mixture (313 mg) gave white needles (55 mg, 26%) (MeOH). The MS [8], PMR [9] and $[\alpha]_D$ [9] of the compound were identical with those of bauerenone.

Fraction B. On acidification with HCl (5%) gave a precipitate which was fractionated with ethyl acetate over silica gel–charcoal (7.2 w/w). The first fraction (400 ml) gave white crystals (MeOH) (867 mg) (substance C), IR (nujol), 3530, 3350, 1690, 810 and 770 cm^{-1} Subsequent fractions were not examined further.

Methyl Esters. Treatment of substance C with diazomethane afforded a mixture of methyl esters as white crystals (MeOH), PMR, δ ($CDCl_3$) 0.66, 0.74, 0.82, 0.84, 0.87, 0.92, 0.94, 0.98, 1.01, 1.06, 1.19, 1.25, 2.23 (d, *J* 10.5 Hz), 2.58 (s), 2.78 (d, *J* 2Hz), 2.97 (d, *J* 9Hz), 3.20 (distorted t, *J* 9Hz), 3.40 (d, *J* 3Hz), 3.16 (s), 3.65 (s), 3.66 (s), 3.96 (m) and 5.27 (m) ppm; MS, *m/e* (% relative abundances in parentheses) 502 (6), 486 (21), 470 (7), 442 (27), 426 (27), 426 (23), 370) (10), 278 (20), 262 (70), 260 (7), 249 (9), 219 (3), 218 (3), 203 (100), 201 (10), 189 (20), 187 (20), 179 (18) and 133 (42).

Methyl Ester Acetates. The mixture of methyl esters was treated with acetic anhydride in dry pyridine to give white crystals (MeOH: H_2O, 1:1 v/v), IR (nujol) 3450, 1740–1730, 1240, 1200, 1030, 830, 790 and 760 cm^{-1}, PMR, δ ($CDCl_3$) 0.64, 0.74, 0.80, 0.88, 0.90, 0.92, 1.06, 1.23, 1.95 (s, CH_3 – CO–O–), 2.03 (s, CH_3 – CO–O–), 3.61 (s, CH_3 – O –), 3.65 (s, CH_3 – O –), 3.66 (s, CH_3 – O –), 4.35 – 4.90 (m) and 5.25 (m, H–C=C–) ppm.

Diospyros mespiliformis *Hochst ex A.DC.*

A mixture of α-amyrin and bauerenol (983 mg) was isolated from dried leaves (322 g) of the plant. The mixture was identified by comparison (TLC, IR and PMR) with the similar mixture from *D. kirkii* leaves. A triterpenoid acid mixture comparable (TLC and IR) with that isolated from *D. kirkii* was also obtained from the leaves of this plant.

Diospyros cornii *Chiov*

The concentrated extract of fresh leaves (1.1 kg) of the plant was extracted with petroleum–ether (40–60°). The petroleum–ether extract gave α-amyrin (1.08 g), m.p. 185°, identified by comparison (TLC, m.p., m.m.p. and IR) with an authentic sample. Lupeol was isolated from the bark of the plant and it was similarly identified. The mother liquor of the leaf extract indicated the presence of lupeol (TLC).

Diospyros usambarensis *F. White*

The concentrated extract from leaves (1.8 kg) of the plant was fractionated through a silica gel– charcoal (7:2% w/w) column with methanol as eluting solvent. The first fraction (200 ml) contained oily substances which were not examined further. The second fraction (350 ml) gave white fine crystals (320 mg) (MeOH). The substance was comparable (TLC, IR, PMR and MS) with the triterpenoid acid mixtures from *D. kirkii* and *D. mespiliformis* leaves.

The chloroform extract of the twigs (70 g) of the plant gave a saturated long chain keto-alcohol as white crystals (EtOH) (50 mg), m.p. 78–80°C, IR (nujol), 3600–3100 (br, –O–H), 1720 (–C=O) and 725 and 715 cm^{-1} $(-CH_2-)_n$ PMR, δ ($CDCl_3$) 0.88 (m, 2 × CH_3), 1.26 $(-CH_2-)_n$, 1.55 (m, $-CH_2-CH_2-CHOH-CH_2-CH_2-$), 2.35 (m, $-CH_2 - CH_2 - CO-CH_2- CH_2-$) and 4.08 (m, $-CH_2-CHOH-CH_2-$) ppm; MS, *m/e* 788 (4.9%) (M^+) and 57 (base peak).

Diospyros consolatae *and* D. verrucosa *Hiern*

A white solid (2.95 g) was isolated from *D. consolatae*

leaves (700 g) and it was identified as a mixture of betulin and betulinic acid by comparison (TLC, IR and m.p.) of the acetates with authentic samples. The triterpenoid (3.25 g), m.p. 295–7°, from leaves of *D. verrucosa* (400 g) was similarly identified as being betulinic acid. Lupeol and α-amyrin were probably present (TLC.) in the leaf and bark extracts of *D. consolatae* while a keto-alcohol related (TLC, m.p., IR and NMR) to that isolated from *D. cornii* was isolated from the plant.

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