gton, collected in Feb. 1987 in Bolivia) was extracted and worked-up as reported previously [7]. CC and TLC afforded 50 mg germacrene D, 9 mg caryophyllene epoxide, 180 mg 7-*o*-methyl sakuranetin, 200 mg sakuranetin, 60 mg naringenin, 1.3 g *ent*-labd-13*E*-ene-8,15-diol and a mixture of acids which were converted to the methyl esters by addition of CH_2N_2. TLC (Et_2O–petrol, 1:1) gave two mixtures. HPLC of one-tenth of the less polar one (RP 8, 100 bar, flow rate, 3 ml/min, MeOH–H_2O, 17:3) afforded 30 mg **1a** (R_t 13.9 min), 50 mg **2a** (R_t 12.3 min) and 30 mg **3a** (R_t 15.0 min). HPLC of one tenth of the second fraction (MeOH–H_2O, 4:1) gave 15 mg **4a** (R_t 17.0 min), 10 mg **5a** and 12 mg **6a**.

The aerial parts (420 g) of *C. tunariensis* (voucher RMK 9634, collected in Feb. 1987 in Bolivia) gave 20 mg germacrene D, 20 mg caryophyllene, 20 mg α-humulene, 900 mg coumarin and 450 mg *o*-hydroxycinnamic acid. Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material.

7β-Acetoxy-6α-angeloyloxy-13,14-dihydrokolavenic acid (**1**). Isolated as its methyl ester **1a**; colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740 (CO_2R); MS *m/z* (rel. int.): 476.314 [M]$^+$ (0.3) (calc. for $C_{28}H_{44}O_6$: 476.314), 416 [M – HOAc]$^+$ (3.3), 316 [416 – RCO_2H]$^+$ (33), 187 (71), 83 [RCO]$^+$ (100).

7β-Acetoxy-6α-isobutyryloxy-13,14-dihydrokolavenic acid (**2**). Isolated as its methyl ester **2a**; colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740/CO_2R; MS *m/z* (rel. int.) 464.314 [M]$^+$ (0.2) (calc. for $C_{27}H_{44}O_6$: 464.314), 404 (2.6), 316 (42), 187 (100), 71 [RCO]$^+$ (51).

7β-Acetoxy-6α-[2-methyl butyryloxy]-13,14-dihydrokolavenic acid (**3**). Isolated as its methyl ester **3a**; colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1735 (CO_2R); MS *m/z* (rel. int.): 478.329 [[M]$^+$ (0.1) (calc. for $C_{28}H_{46}O_6$: 478.329), 418 (1.5), 316 (29), 187 (65), 85 [RCO]$^+$ (31), 57 [85 – CO]$^+$ (100); $[\alpha]_D^{24}$ – 23 ($CHCl_3$; *c* 0.46).

6α,7β-Diacetoxy-13,14-dihydrokolavenic acid (**4**). Isolated as its methyl ester **4a**; colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740 (CO_2R); MS *m/z* (rel. int.): 436 [M]$^+$ (0.3), 376.261 [M – HOAc]$^+$ (1.9) (calc. for $C_{23}H_{36}O_4$: 376.261), 316 (39), 187 (100).

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DITERPENOIDS FROM *DYPTERIX ODORATA*

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Key Word Index—*Dipterix odorata*; Leguminosae; diterpenoids cassane type; structure elucidation; HRGC-MS-C data, 3β-acetoxy vouacapenol.

Abstract—A new cassane diterpene was isolated from the bark of seeds of *Dipterix odorata* together with four known diterpenoids with the same skeleton.

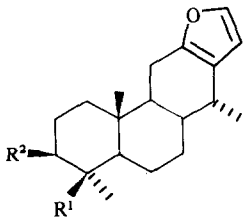
INTRODUCTION

Dipterix odorata Willd (Aubl.) (≡*Coumarouna odorata* Aubl.) belongs to the order Rosales. It is a tall tree (~50 m) indigenous to the Amazonian area, growing in dry plains, known locally as Cumaru or Serrapia in Venezuela. Its seeds are a rich source of coumarin, which is important in the perfumery industry [1].

From the bark and heartwood of this plant earlier workers isolated several isoflavones besides lupeol, betulin and a mixture of methyl esters of fatty acids [2, 3].

RESULTS AND DISCUSSION

Chromatographic fractionation of the petrol extract from the bark of the seed yielded two cassane-type diterpenes, vouacapenic acid (**1**) [4] and β-acetoxy vouacapenol (**2**) which were identified by spectroscopic methods. Chemical transformations performed with vouacapenic acid (**1**) afforded vouacapenol (**3**) and its acetate (**4**) and the hydrocarbon derivative **5** which were identified as minor components in the isolated fractions by HRGC-MS and by co-injection with authentic sam-



	R^1	R^2
1	CO_2H	H
2	CH_2OH	OAc
3	CH_2OH	H
4	CH_2OAc	H
5	Me	H
6	CH_2OTs	H

ples. High resolution mass spectrometry of **2** established the empirical formula $C_{22}H_{32}O_4$ (360.2307, requires 360.2778).

The IR spectrum showed the presence of hydroxyl (ν 3450 cm^{-1}) and carboxyl of acetate (ν 1735 cm^{-1}) functions. The ^1H NMR spectrum of **2** clearly indicated an α,β-substituted furan moiety as shown by two signals at δ6.14 and 7.16. This was corroborated by the mass spectral data, in particular the base peak at *m/z* 108. This corresponds to the fragment resulting from a retro-Diels–Alder rearrangement. The ^1H NMR spectrum of **2** also showed signals for four methyl groups, two on quaternary saturated carbons (δ0.74 and 0.94), one on a tertiary carbon (δ0.92, *d*, J=6 Hz) and the fourth belonging to an acetate function. A double doublet at δ4.86 (J = 10 and 6 Hz), is typical of an acetyl carbinolic proton. The multiplicity of this signal indicates that it is flanked by two hydrogens and, from its coupling constants, an axial orientation can be assigned to it. Consequently, the acetoxy group is equatorial. This limited the possible positions for the acetoxy group to C-1 or C-3. Finally, the presence of an AB quartet at δ3.22 (1H each, *d*, J = 10 Hz) pointed to a hydroxy-methylene moiety.

These data, in combination with comparative analysis of the proton noise decoupled and single frequency off resonance decoupled ^{13}C NMR (Table 1) spectra, allowed expansions to ($MeCO_2CH$–), (■–CH_2OH), substituted furan $(C)_2$, $(CH)_4$ $(CH_2)_5$, $(Me)_3$. These data are consistent with a cassane skeleton substituted in the A ring. The positions of the acetoxyl and hydroxyl groups, located on C-3 and C-19, respectively, were deduced through the comparison of the A-ring carbon shifts of **2** with those of the 3β,19-hydroxy isopimarane diterpenes [5]. Comparison of the B and C rings carbon shifts of **2** with those of the cassane diterpenes confirmed the absence of substituents in these rings [6]. Compound **2** was, thus, identified as 3β-acetoxy vouacapenol.

Table 1. ^{13}C NMR data of compounds **1–3** (25, 2 and 100 MHz $CDCl_3$, TMS as int. standard)

C	1*	2*	3†
1	38.2	36.7	35.2
2	19.6	21.1	18.2
3	29.9	74.5	39.1
4	43.7	42.4	38.3
5	56.2	45.7	55.9
6	31.8	30.6	31.5
7	22.4	22.2	22.2
8	35.7	35.4	35.4
9	45.0	45.1	45.6
10	37.8	37.1	37.1
11	23.3	23.6	21.4
12	149.4	149.0	149.5
13	122.0	122.2	122.3
14	31.4	31.3	31.3
15	109.3	109.4	109.2
16	140.3	140.1	140.8
17	17.5	17.5	17.4
18	29.3	15.2	28.8
19	180.3	65.2	65.3
20	13.4	13.2	15.2

*25.2 MHz.
†100 MHz.

EXPERIMENTAL

Mps: uncorr. CC: Merck silica gel (0.05–0.02 mm); TLC: Merck silica gel H, G or $PF_{254+366}$; NMR 100 and 400 MHz for proton resonance and 25.2 and 100 MHz for carbon resonance; HRGC-MS: Hewlett Packard model 5987A, with H_2 as carrier gas in a 25 m, 0.25 μm, 0.3 mm i.d., glass capillary column.

Isolation of diterpenes **1** *and* **2**. The seeds of *Dipterix odorata* were collected in the Ducke Reserve (Manaus, Brazil). The barks were separated from the seeds and pulverized in a hammer mill. The dust obtained was extracted with petrol for several days. The crude extract was evapd under red. pres. to afford a brownish residue (23.8 g), which was dissolved in MeOH (250 ml). The insoluble material (1.48 g) was filtered off and the filtrate concd. to a vol. of 100 ml. The crude extract (4.0 g) thus obtained was redissolved in $CHCl_3$, absorbed on 4.0 g silica gel 60 and, after evapn of the solvent, placed on top of a column of 100 g of the same absorbent. Elution was started with hexane and the polarity of the eluent increased gradually.

Vouacapenic acid (**1**). Colourless crystals (from MeOH), mp 212–214°. IR ν_{max}^{KBr} cm^{-1}: 3075, 2980, 1690, 1060, and 916; ^1H NMR (100 MHz, $CDCl_3$): δ0.80 (3H, *s*), 0.98 (3H, *d*, J = 6.5 Hz), 1.30 (3H, *s*), 6.44 (1H, *d*, J = 3 Hz), 7.16 (1H, *d*, J = 3 Hz) and 11.46 (1H, *br s*, exchangeable with D_2O); MS *m/z* (rel. int.): 316 $[M]^+$ (30), 301 (8), 255 (6), 131 (20), 108 (100) and 55 (25).

3β-Acetoxy vouacapenol (**2**). Colourless crystals (from hexane–EtOAc, 1:1), mp 154–155°. IR ν_{max}^{KBr} cm^{-1}: 3510, 1735, 1160, 1025, 924 and 890; ^1H NMR (100 MHz, $CDCl_3$): δ0.74 (3H, *s*), 0.92 (3H, *d*, J = 6.5 Hz), 0.94 (3H, *s*), 2.1 (3H, *s*), 3.10 and 3.38 (1H, each, *d*, J = 10 Hz), 4.86 (1H, *dd*, J = 10 and 6 Hz), 6.14 (1H, *d*, J = 3 Hz) and 7.16 (1H, *d*, J = 3 Hz); MS *m/z* (rel. int.): 360.2307 (calc. for 360.2778), 360 $[M]^+$ (30), 345 (2), 300 (5), 270 (20), 131 (25), 108 (100) and 43 (45).

Preparation of vouacapenol (**3**). The acid **1** was methylated by reaction with CH_2N_2 to furnish the ester **1b**. The methyl ester **1b** (350 mg), dissolved in dry THF (5 ml), was refluxed for 3 hr in the presence of $LiAlH_4$ (600 mg). The excess reagent was then destroyed by successive addition of EtOAc, H_2O and 1 M H_2SO_4 (2 ml). The reaction mixture was worked-up in the usual way to furnish, after purification by CC (silica gel), pure alcohol **3** (280 mg).

Vouacapenol (**3**). Colourless crystals (from MeOH), mp 130–131°. IR ν_{max}^{KBr} cm^{-1}: 3450, 3025, 2998, 1470, 1028 and 914; ^1H NMR (400 MHz, $CDCl_3$): δ0.86 (3H, *s*), 0.98 (3H, *d*, J = 6.5 Hz), 1.02 (3H, *s*), 3.12 and 3.44 (1H, each, *d*, J = 10 Hz), 6.16 (1H, *d*, J = 3 Hz) and 7.15 (1H, *d*, J = 3 Hz); MS *m/z* (rel. int.): 302 $[M]^+$ (30), 287 (4), 271 (6), 175 (10), 108 (100) and 55 (25).

Acetylation of vouacapenol (**3**). Ac_2O (2 ml) was added to **3** (50 mg) in pyridine (2 ml). The mixture was left overnight at room temp., extracted with $CHCl_3$ (5 × 25 ml), washed with 1 M HCl, neutralized and dried. Evapn of the solvent *in vacuo* gave a colourless oil (52 mg). IR ν_{max}^{KBr} cm^{-1}: 3025, 2996, 1735, 1465, 1028

and 912; ^{1}H NMR (100 MHz, $CDCl_3$): δ 0.84 (3H, *s*), 0.92 (3H, *s*), 0.98 (3, *d*, $J = 6$ Hz), 3.74 and 3.90 (1H, each, *d*, $J = 10$ Hz), 6.20 (1H, *d*, $J = 2$ Hz) and 7.31 (1H, *d*, $J = 2$ Hz); MS *m/z* (rel. int.): 344 $[M]^+$ (35), 329 (5), 269 (10), 108 (100) and 55 (15).

Tosylation of **3**. Alcohol **3** (50 mg), in dry pyridine (1 ml), was treated with TsCl (90 mg) at room temp. for 1 day. The reaction mixture was worked-up in the usual way, and the crude tosylate **6** purified by preparative silica gel TLC (hexane–EtOAc, 3:1) to afford pure **6** (39 mg). ^{1}H NMR (100 MHz, $CDCl_3$): δ 0.80 (3H, *s*), 0.84 (3H, *s*), 0.98 (3H, *d*, $J = 6$ Hz), 2.50 (3H, *s*), 3.82 and 4.16 (1H, each, *d*, $J = 9$ Hz), 6.16 (1H, *d*, $J = 3$ Hz), 7.15 (1H, *d*, $J = 3$ Hz), 7.36 (2H, *d*, $J = 8$ Hz) and 7.80 (2H, *d*, $J = 8$ Hz).

$LiAlH_4$ *reduction of* **6**. Tosylate **6** (35 mg), in dry THF (2 ml), was refluxed with an excess of $LiAlH_4$ for 15 hr. The product was extracted and purified (prep silica gel TLC hexane–EtOAc, 9:1) to yield pure **5** (22 mg) which was identical (IR, UV, MS and ^{1}H NMR) with an authentic sample.

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TRITERPENOID SAPONINS FROM PLANTS OF ARALIACEAE*

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Key Word Index—*Schefflera impressa*; *Macropanax disperum*; Araliaceae; triterpenoids; triterpenoid glycosides.

Abstract—Two new triterpenoid saponins were isolated from the bark and stem of *Schefflera impressa* C. B. Clarke and characterized as 3β,23-dihydroxy-urs-12-en-28-oic acid-3-*O*-β-D-glucuronopyranoside 6′-*O*-methyl ester and 4-epihederagenin-3-*O*-β-D-glucuronopyranoside-6′-*O*-methyl ester alongwith oleanolic acid; hederagenin; 23-hydroxy ursolic acid and hederagenin-3-*O*-β-D-glucuronopyranoside-6′-*O*-methyl ester. While leaves of *Macropanax disperum* Blume led to the isolation of 3β-hydroxy-olean-12-en-28-*O*-β-D-glucopyranoside; oleanolic acid and hederagenin.

INTRODUCTION

Schefflera impressa C. B. Clarke (Napalese name 'Balu Chinia') and *Macropanax disperum* Blume are evergreen woody trees of the family Araliaceae, which are distributed in the Himalayas from Kumaun to Bhutan. No work has been done on the constituents of these plants, however. As a part of our studies on Indian medicinal plants for their biological active saponins [1] these plants have now been examined. The present communication describes the isolation and structure elucidation of two new triterpenoid glycosides.

RESULTS AND DISCUSSION

The stem and bark of *S. impressa* and leaves of *M. disperum* were extracted with methanol. The water soluble portion of the methanol extract was successively extracted with hexane, benzene, chloroform, ethyl acetate and butanol. The butanol-soluble fraction of *S. impressa* was subjected to repeated vacuum liquid chromatography (VLC) [2] over TLC silica gel to give sapogenin SI-A (**1**, **2**) saponins SI-B (**2a**) and C (**3**, **4**). On TLC SI-A, B and C showed a single spot and could not be resolved in several solvent systems.

Isomeric mixtures of olean-12-ene and urs-12-enes as well as hederagenin and its isomer 4-epihederagenin [3] have frequently been isolated from the plant kingdom. Separation of these triterpenoids is a challenging problem that remain unsolved. However ^{13}C NMR spectroscopy permits their identification [3–5].

Sapogenin SI-A obtained from *S. impressa* contained the triterpenes **1** and **2**. On the basis of ^{13}C NMR analysis of SI-A, they were identified as hederagenin (**1**) and 3β,23-dihydroxy-urs-12-en-28-oic acid (**2**) [4]. This is the second report of the isolation of this isomeric mixture from nature.

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