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ACIDIC DAMMARANE ARABINOFURANOSIDES FROM *COMBRETUM ROTUNDIFOLIUM*

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Key Word Index — *Combretum rotundifolium*; South American Combretaceae; acid dammarane arabinofuranoside; (16*R*)-16-*O*-L-arabinofuranosyl-3β-hydroxydammara-20,24-dien-29-oic acid; 17-octanordammarane; (16*R*)-16-*O*-α-L-arabinofuranosyl-3β-hydroxymansumbin-13-en-28-oic acid.

Abstract—An acidic dammarane arabinofuranoside and its 17-octanor equivalent have been isolated from the leaves of the South American liana, *Combretum rotundifolium*. Spectral analysis has identified these two compounds as (16*R*)-16-*O*-α-L-arabinofuranosyl-3β-hydroxydammara-20,24-dien-29-oic acid and the mansumbinane, (16*R*)-16-*O*-α-L-arabinosyl-3β-hydroxymansumbin-13-en-28-oic acid.

INTRODUCTION

As an extension of the chemotaxonomic investigation of the African *Combretum* [1], the chemical constituents of three South American species, *C. laxum*, *C. rotundifolium* and *C. fruticosum* were examined. Impetus for this study was provided by the recent isolation from the South American species, *C. leprosum*, of the cycloartenoid mollic acid previously found only in several African *Combretum* species [2]. This discovery presents intriguing phytochemical and phytogeographic questions related to the ancestry and development of this genus on the two continents.

The mixtures of triterpenoid acids and their glycosides previously isolated from the leaves of African *Combretum* were found to be secreted through scale-like trichomes on to the surface of the leaf where they form a surface coating. Both the composition of this predominantly acidic triterpenoid glycoside coating and the morphology of the scales have been found to be species specific [3]. Although scales were present on all three South American species, only leaves from *C. rotundifolium* and *C. fruticosum* yielded acidic triterpenoid compounds when extracted with bicarbonate solution in the usual way [3]. *Combretum fruticosum* gave a low yield of a complex mixture that proved difficult to separate, whereas the liana, *C. rotundifolium*, which has been used for medicinal purposes by the Karijonas Indians [4], yielded two new dammarane arabinofuranoside compounds as the major constituents of the surface coating. One of these compounds was given the trivial name rotundifolic acid and the other is a representative of the mansumbinanes or octanordammaranes previously isolated from *Commifera incisa* resin [5].

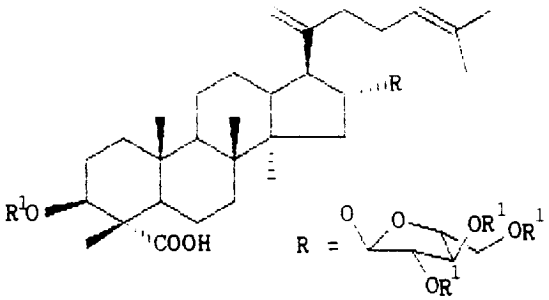
RESULTS AND DISCUSSIONS

The two major and also most polar constituents of the leaf coating were obtained pure by repeated flash column chromatography.

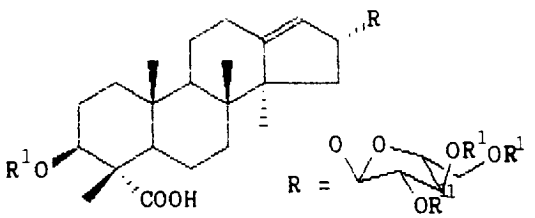
The molecular formula of rotundifolic acid (**1**), the most abundant and least polar of the two, was established as $C_{35}H_{56}O_8$ (by HR-FABMS and EIMS of the peracetate). The presence of a carboxylic acid function, six methyl groups, two double bonds (one di- and the other tri-substituted) and four hydroxy groups, of which three formed part of a sugar moiety, was indicated by NMR spectroscopy; the pattern of the ^{13}C NMR resonances for the aglycone carbons suggested a dammarane skeleton for the compound (Table 1).

The sugar, shown to be a pentose by the presence of major peaks at *m/z* 259 (base peak) and 139 in the EIMS of the peracetate **1a** [8], was identified as L-arabinofuranose by ^{13}C NMR [9]. The anomeric proton appears as a singlet at δ5.32 in the ^{1}H NMR spectra of **1** and **1a**, which shows that this sugar is attached to the aglycone via an α-linkage. This complies with the general rule that in nature the glycosidic linkages of sugars in the D-and L-series are β and α, respectively [10].

Characteristic methyl resonances at δ1.59 and 1.66 plus an olefinic proton at δ5.09 placed the trisubstituted double bond in an isopropylidene moiety on a C-17 side chain. The absence of a methyl doublet for C-21 and the presence of resonances typical of an endomethylene function [^{1}H NMR of **1**: δ4.83 and 4.86 (2H, 2 *s*) and ^{13}C NMR: δ108.4 (*t*) and δ150.4 (*s*) [11, 12], places the second double bond at C-20 (21) where its anisotropic effects cause significant shielding of the βH-16 and deshielding of the H-17 protons. A 20,25-diene side chain is



	R^1
1	H
1a	Ac



	R^1
2	H
2a	Ac

not uncommon in dammarane type triterpenoids, e.g. **3** [13]. The unusually high field resonance (δ11.6) of one of the four remaining methyl resonances in the ^{13}C NMR spectrum and its deshielded position (δ1.52) in the ^{1}H NMR spectrum of 1, suggested that it was *geminal* to the carboxy function. Biogenetic evidence from the African *Combretum* (e.g. mollic and imberbic acids) and the absence of resonances for the two *geminal* methyls (C-28 and C-29) places this grouping at C-4 with the carboxy function at C-28 and the shielded methyl at C-29 [2]. The three remaining angular methyls have ^{13}C NMR resonances at δ15.9, 16.5 and 16.9, which are consistent with methyls belonging to the dammarane skeleton [14].

Apart from the side chain, a comparison of the ^{13}C NMR chemical shifts of compounds **1** and **1a** with those of standard dammarane compounds [14], shows that differences occur in rings A and D. In ring A these differences can be attributed to the presence of the C-28 carboxy substituent and this was confirmed by the excellent agreement between the ^{13}C NMR chemical shifts in rings A and B of **1** and 3β-hydroxyolean-12-en-23,28-dioic acid (**4**) (Table 1). Acetylation of **1** produced typical acetylation shifts for the C-3 hydroxyl function (δ75.0); therefore, the carbon at δ80.8 unaffected by acetylation

Table 1. ^{13}C NMR spectral data for compounds **1–3**

C	4* [6, 7]	1†	1a*	2†	2a*	3*
1	39.0	39.1	38.4	38.9	38.4	39.0
2	28.2	27.6	23.1	27.4	23.1	27.5
3	75.4	75.0	77.3	74.8	77.2	79.0
4	54.4	54.3	52.0	54.1	52.0	39.2
5	51.9	52.0	51.4	51.8	51.4	56.0
6	21.6	21.1	21.1	21.1	21.1	18.3
7	33.2	35.1	34.8	34.9	34.8	35.4
8	40.1	40.5	40.6	40.3	40.6	40.5
9	48.3	50.5	50.4	50.3	50.4	50.8
10	36.8	36.4	36.7	36.3	36.6	37.2
11		21.3	21.3	21.0	21.3	21.4
12		24.5	24.3	24.3	24.4	25.0
13		43.9	44.4	130.8	130.9	47.6
14		48.1	48.4	47.9	48.4	49.5
15		37.5	37.5	37.5	37.7	31.4
16		80.8	81.0	80.8	81.4	29.4
17		55.2	55.2	128.1	128.8	45.4
18	17.3	16.3	16.5	16.2	16.5	15.9
19	16.0	15.5	15.9	15.3	15.9	15.7
20		150.4	150.0	—	—	152.6
21		108.4	109.0	—	—	107.6
22		34.8	34.7	—	—	34.3
23		26.7	26.7	—	—	27.0
24		124.8	124.2	—	—	124.6
25		130.7	131.6	—	—	131.4
26		25.3	25.7	—	—	25.7
27		17.4	17.7	—	—	17.7
28	180.6	180.1	180.0	180.0	180.7	28.6
29	12.2	11.6	11.5	11.4	11.4	16.2
30		16.7	16.9	16.5	16.9	15.4
1′		106.8	103.7	106.8	103.9	
2′		83.5	80.2	83.3	80.2	
3′		78.2	77.2	78.1	77.1	
4′		84.9	81.5	84.6	81.5	
5′		62.0	63.4	62.0	63.4	

Assignments based on DEPT and HETCOR ^{13}C–^{1}H experiments.

*Measured in $CDCl_3$.

†Measured in pyridine-d_5.

belongs to the glycoside linkage and must be attached to ring D at C-15 or C-16. The sugar moiety was located at the latter carbon for the following reason. The deshielding effect of the C-20 double bond causes the H-17 proton to resonate at the unusually low field position of δ2.30 in the ^{1}H NMR spectrum of **1a** (δ2.47 for **1**) where it is clearly visible as a sharp doublet of doublets (J_1 = 12 Hz, J_2 = 6 Hz). This splitting must be due to the H-17 proton coupling with the proton on C-13 and a single H-16 proton. If the sugar was placed at C-15, coupling with a second C-16 proton would result in a far more complex splitting pattern for the H-17 proton.

The assignment of the *R*-configuration to C-16 was based on ^{1}H NMR data. Firstly, to explain the unusually highfield resonance of the H-16 proton (δ4.02 in the ^{1}H NMR of **1** and δ4.28 in **1a**) it has to extend into the shielding zone of the C-20 double bond and must, there-

fore, have the β-configuration. Secondly, the H-16 resonance in **1** and **1a** does not correspond with published values for an αH-16 proton. Whereas this signal is reported as a double triplet (J = 5 Hz and J = 5.5 Hz) at δ4.5 in a 16(*S*), 20(*R*)-dihydroxydammarane from *Boswellia freerana* [15], the signal attributed to H-16 in the ^{1}H NMR spectra of **1** and **1a** is quite different, appearing instead as a broad triplet. Finally, the α-configuration for the sugar moiety is sterically the most favourable position. Consequently, the *R*-configuration was assigned to C-16 and the structure of **1** is proposed as (16*R*)-16-*O*-α-L-arabinofuranosyl-3β-hydroxydammara-20,24-dien-29-oic acid. Although dammarane compounds oxygenated at C-16 are common, and α-L-arabinofuranoside moieties and 20,24-diene side chains have been reported before, this is the first time a dammarane with a C-28 carboxy function has been isolated [8, 14, 15].

The structural elucidation of compound **2**, molecular formula $C_{35}H_{50}O_{12}$ by EIMS of the peracetate **2a**, was relatively straightforward, although the isolation of the compound was difficult. In the ^{13}C NMR spectra (Table 1), the only difference between compounds **1** and **2** is that the side chain carbons are absent from **2** and the C-13 and C-17 resonances have shifted. In addition, the signals for the H-21 protons and the isopropylidene function are missing from the ^{1}H NMR spectra of **2** and **2a**. The loss of the side chain was confirmed by mass spectrometry. Thus, the molecular mass of **2a** differs from **1a** by the equivalent of a C_8H_{14} side chain. Compound **2** therefore corresponds to the octanordammarane mansumbinanes from *Commiphora incisa* resin [5].

However, the double bond introduced in **2** cannot be at C-16(17), because of the presence of the C-16 sugar moeity. Since the ^{13}C NMR chemical shifts of C-13 (δ43.9) and C-17 (δ55.2) were absent, the two new olefinic carbon resonances at δ128.1 and 130.8 in the spectra of **2** and at δ128.8 and 130.9 in the spectra of **2a** were assigned to C-13 and C-17. The structure of **2** is, therefore, (16*R*)-16-*O*-α-L-arabinofuranosyl-3β-hydroxymansumbin-13-en-28-oic acid. In ref. [5] mansumbinane was incorrectly named as a gonane and not as an 18-norandrostane (Rule 2S-2.2 IUPAC-IUB 1971. Definitive Rules for Steroid Nomenclature).

As in the case of the mansumbinanes, **2** must be derived from a compound such a rotundifolic acid by fission of the C-17/C-20 bond with concomitant formation of the C-13 (17) double bond. On standing, **2** degrades readily into an intractable mixtures, which is shown by ^{13}C NMR to contain additional double bonds due to the loss of the sugar. This lability, which makes it difficult to isolate pure, must be due to the proximity of the double bond to the sugar moiety.

EXPERIMENTAL

General. IR: KBr discs; ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR: $CDCl_3$ or pyridine-d_5, using TMS as int. standard; EIMS (probe): 70 eV; FAB-MS: glycerol matrix (positive ion mode).

Plant material. Leaves were collected in January 1993 from plants growing in the Botanic Gardens, Rio de Janeiro, Brazil.

Extraction and isolation of the leaf coating. The air-dried leaves (315 g) were submerged in 4 l aq. 1% $NaHCO_3$, gently warmed for 2 hr and the strongly effervescing soln left overnight at 35°. The soln was decanted and acidified with conc HCl and the resulting precipitate collected by gravity filtration and washed thoroughly to remove excess of acid. The dried ppt. (20.7 g), which consisted of the triterpenoid acids and a large quantity of polar, polymeric material, was extracted with $CHCl_3$–EtOH (1:1). Repeated silica gel CC (eluent: $CHCl_3$–MeOH–H_2O, 12:3:1 lower phase) of this extract (5 g) afforded **1** (56 mg) and **2** (15 mg).

Rotundifolic acid (**1**). Amorphous solid, FAB-MS: *m/z* 605 $[M+1]^+$; $C_{35}H_{56}O_8$ requires $[M]^+$ 604; IR ν_{max} cm^{-1}: 3424 (OH), 2944, 2880, 2650 (CO_2H dimer), 1699 (carboxy C=O), 1638, 1522, 1465, 1449, 1388, 1078, 1043, 995, 956, 889; ^{1}H NMR (pyridine-d_5): δ5.32 (1H, *br s*, H-1′), 5.17 (1H, *t*, H-24), 4.86 and 4.83 (2H, 2×*s*, 2×H-21), 4.73–4.65 (2H, *m*, H-2′, 3′), 4.56–4.47 (2H, *m*), 4.27 (1H, *br t*, H-16), 4.22–4.06 (2H, *m*, H-4′, 5′), 2.47 (1H, *dd*, J_1 = 12 Hz; J_2 = 6 Hz, H-17), 1.52 (6H, *s*, Me-26, 28), 1.47 (3H, *s*, Me-27), 0.95 (3H, *s*), 0.79 (3H, *s*), 0.77 (3H, *s*); ^{13}C NMR: Table 1.

Rotundifolic acid tetraacetate (**1a**). Acetylation of **1** (15 mg) was carried out in Ac_2O–pyridine and worked up in the usual way to give a glass (16 mg). Found: $[M]^+$ *m/z* 772.4380; $C_{43}H_{64}O_{12}$ requires 772.4381. EIMS *m/z* (rel. int.): 772 $[M]^+$ (1), 712 $[M-HOAc]^+$ (1.5), 652 $[M-2xHOAc]^+$ (1), 609 (1.5), 512 $[M-sugar]$ (1.5), 496 (2), 453 $[M-sugar-HOAc]^+$ (1), 429 (3), 392 (10), 368 (3), 327 (5) 283 (15), 259 [sugar] (100), 217 (20), 203 (5), 175 (9), 173 (7), 157 (17), 139 [sugar] (90); ^{1}H NMR ($CDCl_3$): δ5.14 (1H, *dd*, J_1 = 11.5 Hz, J_2 = 4.5 Hz, H-3), 5.09 (1H, *m*, H-24), 4.97 (1H, *br s*), 4.95 (1H, *s*, H-1′), 4.90 (1H, *d*), 4.76 (2H, *br s*, 2xH-21), 4.36 (1H, *d*, H-3′), 4.18–4.12 (2H, *m*, H-4′, 5′), 4.02 (1H, *br t*, H-16), 2.30 (1H, *dd*, J_1 = 12 Hz, J_2 = 6 Hz, H-17), 2.07 (3H, *s*, OCOMe), 2.06 (3H, *s*, OCOMe), 1.98 (3H, *s*, OCOMe), 1.66 (3H, *s*, Me-26), 1.59 (3H, *s*, Me-27), 1.17 (3H, *s*, Me-29), 1.04 (3H, *s*, Me), 0.91 (3H, *s*, Me), 0.87 (3H, *s*, Me); ^{13}C NMR: Table 1.

Compound **2**. Amorphous powder (15 mg). ^{13}C NMR: Table 1.

Compound **2a**. Acetylation of **2** (12 mg) was carried out in Ac_2O and pyridine (r.t. 24 hr) to give **2a** (15 mg) as an amorphous solid. EIMS *m/z* (rel. int.): 662 $[M]^+$ (1), 647 (1), 568 (1), 554 (4), 540 (4), 524 (8), 512 (7), 496 (9), 481 (7), 470 (5), 386 (10), 371 (9), 368 (6), 327 (9), 300 (9), 259 [sugar] (95), 233 (15), 220 (29), 203 (16), 187 (16), 175 (39), 173 (49), 157 (48), 143 (94), 139 [sugar] (100); IR ν_{max} cm^{-1}: 2944, 2880, 2650 (CO_2H dimer), 1744 (acetate C=O), 1699 (carboxy C=O), 1641, 1452, 1369, 1239 (acetate C–O–C), 1072, 1040, 1004, 969, 889, 806; ^{1}H NMR ($CDCl_3$): δ5.59 (1H, *m*, H-17), 5.14 (1H, *dd*, J_1 = 12 Hz, J_2 = 4 Hz, H-3), 4.98–4.91 (2H, *m*, H-2′, 3′), 4.94 (1H, *br s*, H-1′), 4.78 (1H, *m*), 4.41–4.00 (3H, *m*, H-16, 4′, 5′), 2.07

(6H, 2 × *s*, OCOMe), 1.98 (6H, 2 × *s*, OCOMe), 1.32 (3H, *s*, Me-29), 1.04 (3H, *s*, Me), 0.91 (3H, *s*, Me), 0.87 (3H, *s*, Me).

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