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PENTACYCLIC TRITERPENES FROM *MYRIANTHUS LIBERECUS*

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Key Word Index—*Myrianthus liberecus*; Cecropiaceae; arjunolic acid; 3-isoarjunolic acid; 3*β*-*O*-*E*-coumaroylarjunolic acid; pentacyclic triterpenes.

Abstract—From the methylated trunk wood extracts of *Myrianthus liberecus*, six pentacyclic triterpenes have been isolated as their methyl esters. These included the known methyl benthamate, methyl euscaphate, methyl tormentate, methyl arjunolate, methyl 3-isoarjunolate and methyl 3*β*-*O*-(4″-*O*-methyl-*E*-coumaroyl)-arjunolate, a new triterpene derivative.

INTRODUCTION

Myrianthus liberecus is a small tree of the African rain forest [1]. The genus is represented in tropical African forests by seven species, four of them being found in Cameroon: *M. arboreus*, *M. liberecus*, *M. serratus* and *M. preussii* [2]. Previous phytochemical studies of two Cameroonian Cecropiaceae, *M. arboreus* and *Musanga cecropioides*, led to the isolation and identification of a number of pentacyclic triterpenes [3–11]. In a continuation of our studies on this family, we examined the trunk wood components of *M. liberecus*. We now report on the isolation and characterization from the methylated extracts of this plant material of the methyl esters of the pentacyclic triterpenoids arjunolic acid (**1**), 3-isoarjunolic acid (**2**) and the new derivative, 3*β*-*O*-*E*-coumaroylarjunolic acid (**3**).

RESULTS AND DISCUSSION

The ethyl acetate extract of the defatted trunk wood of *M. liberecus* was methylated and yielded, after vacuum liquid chromatography (VLC) and crystallization, six methyl esters of pentacyclic triterpenoid acids; methyl tormentate [8, 11], methyl euscaphate [11], methyl benthamate [12], methyl arjunolate (**1**) [13–17], methyl 3-isoarjunolate (**2**) [18–21] and a new derivative methyl 3*β*-*O*-*p*-methoxy-cinnamoyloxy-2*α*,23-dihydroxyolean-12-en-28-oate (**3**). NMR assignments for **1** and **2** were based on the 2D NMR data (Tables 1 and 2).

The EI mass spectrum of **3** displayed a $[M]^+$ at m/z 662, compatible with the molecular formula $C_{41}H_{58}O_7$. The IR spectrum exhibited absorption bands at 3330 (alcohol), 1720 (ester), 1620 and 1565 cm^{-1} (trisubstituted double bond and aromatic ring). Interpretation of the ^1H and ^{13}C spectral data by means of ^1H–^1H, ^1H–^{13}C COSY and ^1H–^{13}C long-range COSY experiments, and comparison with methyl arjunolate, allowed full assignment of all of the NMR signals and led to structure **3**.

The ^1H NMR spectrum of **3** (Table 1) was close to that of **1**, but differed by the downfield shift of H-3 which was at δ_H 4.80 instead of 3.39 and the presence of additional signals for ethylenic protons at δ_H 6.34 (H-2′) and 7.69 (H-3′) with a mutual coupling (15.9 Hz) characteristic of a *trans* configuration, and the aromatic protons of a *p*-disubstituted benzene ring, suggesting the presence of a *p*-substituted *E*-cinnamoyl moiety. This substituent was placed on C-3, because of the downfield effect observed on H-3. Since, the respective coupling patterns of H-2 and H-3 remained unchanged as compared with those of **1**, the *p*-substituted cinnamoyl group was situated in the 3*β*-position. The ^{13}C NMR spectrum of **3** exhibited signals at δ_C 121.9 (C-12) and 144.0 (C-13) compatible with the Δ^{12}-oleanene type skeleton, and signals at δ_C 66.6 and 79.9 assigned to C-2 and C-3, respectively. Additional signals not found in the spectrum of **1** were those of a 4-*O*-methyl-*E*-coumaroyl moiety (Tables 1 and 2).

Structure **3** was further supported by a ^1H–^{13}C long-range COSY experiment, optimized for a coupling constant value of 7 Hz: cross-peaks were observed between the coumaroyl carbonyl (δ_C 169.1, C-1′) and H-3 (δ_H 4.80) and both vinylic protons, H-2′ (δ_H 6.34) and H-3′ (δ_H 7.69). Cross-peaks were also observed between C-3 at δ_C 79.9 and CH_3-24 at δ_H 0.72 and the hydroxymethylene protons at δ_H 2.91 and 3.35. Moreover, the structure of the

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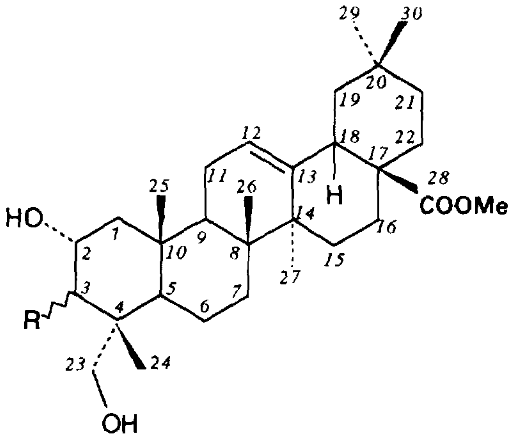

R

1: β-OH

2: α-OH

3: β-*p*-MeO-C_6H_5-CH = CH-CO-O-

coumaroyl moiety was confirmed by the correlations protons C-1″ and δ_C 126.8 and the vinylic protons H-3′ and H-2′, as well as protons H-2″ and H-6″ at δ_H 6.89. Correlations were also observed between C-4″ at δ_C 161.7 and protons at δ_H 3.82 (OCH_3) and 7.47 (H-3″ and H-5″). The fragmentation ions in the EI mass spectrum agreed with those of structure **3**, showing the base peak at *m/z* 161 due to the 4-*O*-methyl-coumaroyl moiety. The above evidence established the structure of **3**, and the related natural acid was thus 3β-*O*-*E*-coumaroylarjulonic acid, as no methoxyl group was detected in the unmethylated fraction. Recently, the related compound 3β-*O*-coumaroylmaslinic acid has been isolated from *Leptospermum scoparium* (Myrtaceae) [22].

EXPERIMENTAL

General. NMR: 300 MHz (^1H) and 75 MHz (^{13}C), chemical shifts of coupled protons measured either from 1D or from 2D COSY spectra for complex entangled systems, with TMS as int. standard.

Table 1. ^1H NMR data for compounds **1**–**3** ($CDCl_3$)

C	**1**	**2**	**3**
1ax	0.88 *m*	1.23 *m*	0.87 *m*
eq	1.95 *m*	1.63 *m*	2.08 *dd* (12.5, 4.3)
2ax	3.73 *ddd* (12.0, 10.0, 3.9)*	3.95 *ddd* (12.0, 4.4, 2.7)	4.05 *ddd* (10.6, 9.8, 4.3)
3ax	3.39 *d* (10.0)	—	4.80 *d* (9.8)
eq	—	3.64 *d* (2.7)	—
5	1.05 *m*	1.58 *m*	1.05 *m*
6	1.37 *m*	1.34 *m*	1.52 *m*
7ax	1.49 *m*	1.49 *m*	1.47 *m*
eq	1.24 *m*	1.25 *m*	1.24 *m*
9	1.62 *m*	1.72 *m*	1.61 *m*
11	1.91 *m*	1.91 *m*	1.91 *m*
12	5.26 *dd* (3.7, 3.6)	5.27 *dd* (3.5, 3.5)	5.27 *dd* (3.7, 3.6)
15ax	1.57 *m*	1.57 *m*	1.58 *m*
eq	1.05 *m*	1.05 *m*	1.06 *m*
16ax	1.90 *m*	1.91 *m*	1.91 *m*
eq	1.62 *m*	1.58 *m*	1.59 *m*
18	2.83 *dd* (13.3, 4.5)	2.84 *dd* (13.7, 4.0)	2.85 *dd* (13.9, 3.3)
19ax	1.59 *m*	1.57 *m*	1.59 *m*
eq	1.14 *m*	1.14 *m*	1.13 *m*
21ax	1.34 *m*	1.33 *m*	1.34 *m*
eq	1.14 *m*	1.14 *m*	1.14 *m*
22ax	1.66 *m*	1.64 *m*	1.66 *m*
eq	1.49 *m*	1.49 *m*	1.50 *m*
23a	3.62 *d* (11.7)	3.51 *d* (11.2)	2.91 *d* (12.7)
23b	3.39 *d* (11.7)	3.46 *d* (11.2)	3.35 *d* (12.7)
24	0.83 *s*	0.70 *s*	0.72 *s*
25	0.99 *s*	0.95 *s*	1.04 *s*
26	0.69 *s*	0.69 *s*	0.71 *s*
27	1.10 *s*	1.13 *s*	1.13 *s*
29	0.87 *s*	0.87 *s*	0.88 *s*
30	0.90 *s*	0.90 *s*	0.91 *s*
28-OMe	3.59 *s*	3.60 *s*	3.60 *s*
2′	—	—	6.34 *d* (15.9)
3′	—	—	7.69 *d* (15.9)
2″,6″	—	—	6.89 *m*
3″,5″	—	—	7.47 *m*
4″-OMe	—	—	3.82 *s*

**J* (Hz) in parentheses.

Table 2. ^{13}C NMR data for compounds **1**–**3** ($CDCl_3$)

C	1	2	3
1	46.0	41.3	46.7
2	68.7	66.6	66.6
3	80.1	78.6	79.9
4	42.5	41.1	43.7
5	48.8	42.1	46.6
6	18.3	17.8	17.8
7	32.2	32.1	32.4
8	39.3	39.4	39.4
9	47.5	47.3	47.5
10	38.2	38.0	38.0
11	23.4	23.4	23.5
12	122.1	122.0	121.9
13	143.8	144.0	144.0
14	41.7	41.7	41.8
15	27.6	27.6	27.6
16	23.0	23.0	23.0
17	46.7	46.7	46.7
18	41.2	41.2	41.3
19	45.8	45.8	45.8
20	30.7	30.7	30.7
21	33.8	33.8	33.8
22	32.3	32.3	32.2
23	69.9	71.3	64.6
24	12.8	17.4	13.8
25	17.0	16.7	17.3
26	16.9	16.9	16.9
27	26.0	26.1	26.0
28	178.3	178.3	178.2
29	33.1	33.1	33.1
30	23.6	23.6	23.6
28-OMe	51.5	51.5	51.5
1′	—	—	169.1
2′	—	—	114.5
3′	—	—	146.0
1″	—	—	126.8
2″,6″	—	—	130.0
3″,5″	—	—	114.4
4″	—	—	161.7
4″-OMe	—	—	55.4

Plant material. The trunk wood of *M. liberecus* P. Beauv. was collected at Mount Kala (Yaoundé zone, Cameroon) in June 1991. A voucher specimen (92 DL) is deposited at the National Herbarium (Yaoundé, Cameroon).

Extraction and isolation. The air-dried and pulverized trunk wood of *M. liberecus* (6.4 kg), was macerated at room temp. in MeOH (20 l) for 72 hr and the operation repeated × 3. The combined MeOH extracts were concd to dryness, defatted with *n*-hexane and dissolved in EtOAc to yield, after evapn of the solvent, a brown gum (48 g). The latter was further methylated with MeI in an Me_2CO dispersion of dry K_2CO_3 for 24 hr with refluxing. Repetitive VLC of the resulting mixt. on Silica gel 60 and elution with *n*-hexane–EtOAc with increasing amounts of EtOAc, afforded a mixt. of sterols and then methyl benthamate (20 mg), methyl tormentate (250 mg), methyl euscaphate (50 mg), methyl arjunolate (**1**, 70 mg), methyl 3-isoarjunolate (**2**, 35 mg) and the new *p*-methoxycinnamoyl derivative **3** (30 mg).

Methyl 2α,3β,22-trihydroxyolean-12-en-28-oate (*methyl arjunolate*) (**1**). $C_{31}H_{50}O_5$, crystals, mp 249–251° (*n*-hexane–EtOAc), lit. 248–250° [16], 227–237° [17]. IR, ν_{max}^{KBr} cm^{-1}: 3400, 2952, 2830, 1730, 1640, 1460, 1360–1380, 1240, 1220, 1160, 1140, 985, 935, 825, 745, 690; EIMS (70 eV, 200°) *m/z* (rel. int.): 502 $[M]^+$ (1), 485 (0.5), 467 (1), 444 (1), 425 (0.5), 405 (1), 309 (1), 262 (RDA, 30), 249 (9), 215 (6), 203 (100), 189 (24), 187 (11), 173 (10), 133 (17), 119 (10), 105 (8), 95 (5), 69 (7), 56 (9).

Methyl 2α,3α,22-trihydroxyolean-12-en-28-oate (*methyl 3-isoarjunolate*) (**2**). $C_{31}H_{50}O_5$, amorphous powder. IR, ν_{max}^{KBr} cm^{-1}: 3445, 3638, 2953, 2928, 2859, 1714, 1645, 1460, 1260, 1215, 1165, 1142, 987, 935, 823, 746, 691, 656; EIMS (70 eV, 200°) *m/z* (rel. int.): 502 $[M]^+$ (3), 469 (1), 442 (2), 425 (1), 407 (1), 393 (1), 377 (1), 309 (1), 262 (RDA, 82), 249 (8), 203 (RDA-AcOH, 100), 189 (26), 173 (15), 133 (18), 119 (18), 107 (10), 105 (14), 95 (13), 69 (9), 55 (11).

Methyl 3β-O-p-methoxy-E-cinnamoyloxy-2α,23-dihydroxyolean-12-en-28-oate (*methyl 3β-O-[4″-O-methyl-E-coumaroyl]-arjunolate*) (**3**). $C_{41}H_{58}O_7$, crystals, mp 262–264° (*n*-hexane–EtOAc); IR ν_{max}^{KBr} cm^{-1}: 3330, 2950, 1750, 1680, 1620, 1565, 1515, 1460, 1365–1390, 1330, 1310, 1260, 1170, 1130, 1085, 1050, 1020, 960, 825; EIMS (70 eV, 200°) *m/z* (rel. int.): 662 $[M]^+$ (1), 603 (1), 502 (1), 466 (2), 442 (2), 424 (1), 407 (4), 401 (4), 262 (46), 203 (93), 189 (21), 161 (100), 133 (33), 119 (15), 105 (10), 81 (14), 69 (12).

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REFERENCES

1. Berg, C. C. (1978) *Taxon* **27**, 39.
2. Berg, C. C., Higmann, M. E. E. and Weerdenburg, J. C. A. (1985) *Flore du Cameroun* (Satabié, B. ed.), Vol. 28, pp. 262–270. MESRES, Yaoundé, Cameroon.
3. Lontsi, D., Sondengam, B. L. and Ayafor, J. F. (1989) *J. Nat. Prod.* **52**, 52.
4. Lontsi, D., Sondengam, B. L., Ayafor, J. F., Tsoupras, G. and Tabacchi, R. (1990) *Planta Med.* **56**, 287.
5. Lontsi, D., Sondengam, B. L., Martin, M. T. and Bodo, B. (1991) *Phytochemistry* **30**, 1621.
6. Lontsi, D., Sondengam, B. L., Martin, M. T. and Bodo, B. (1991) *Phytochemistry* **30**, 2361.
7. Lontsi, D., Sondengam, B. L., Martin, M. T. and Bodo, B. (1992) *Phytochemistry* **31**, 4285.
8. Lontsi, D., Sondengam, B. L., Ayafor, J. F. and Connolly, J. D. (1987) *Tetrahedron Letters* **28**, 6683.
9. Ngounou, F. N., Lontsi, D., Ayafor, J. F. and Sondengam, B. L. (1987) *Phytochemistry* **26**, 3080.
10. Ngounou, F. N., Lontsi, D. and Sondengam, B. L. (1988) *Phytochemistry* **27**, 301.

11. Ngounou, F. N., Lontsi, D. and Sondengam, B. L. (1988) *Phytochemistry* **27**, 2287.
12. Bermejo, J., Breton, J. L., de la Fuente, G. and Gonzales, A. G. (1967) *Tetrahedron Letters* **47**, 4649.
13. Mahato, S. B. and Kundu, A. P. (1994) *Phytochemistry* **37**, 1517.
14. Ojinnaka, C. M., Okogun, J. I., Okorie, D. A. (1984) *Phytochemistry* **23**, 1125.
15. Furuya, T., Orihara, Y. and Hayashi, C. (1987) *Phytochemistry* **26**, 715.
16. Diallo, B., Vanhaelen, M., Vanhaelen-Fatre, R., Konoshima, T., Kozuka, M. and Tokuda, H. (1989) *J. Nat. Prod.* **52**, 879.
17. King, F. E., King, T. J. and Rose, J. M. (1954) *J. Chem. Soc.* 3995.
18. Bowden, B. F., Cambie, R. C. and Parnell, J. C. (1975) *Aust. J. Chem.* **28**, 91.
19. Kojima, H., Tominaga, H., Sato, S. and Ogura, H. (1987) *Phytochemistry* **26**, 1107.
20. Kojima, H. and Ogura, H. (1989) *Phytochemistry* **28**, 1703.
21. De la Torre, M. C., Bruno, M., Piozzi, F., Savona, G., Rodriguez, B. and Arnold, N. A. (1990) *Phytochemistry* **29**, 668.
22. Häberlein, H. and Tschiersch, K.-P. (1994) *Phytochemistry* **35**, 765.