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CHEMICAL STUDIES ON THE CECROPIACEAE: A NOVEL A-RING SECO TRITERPENE FROM *MUSANGA CECROPIOIDES*

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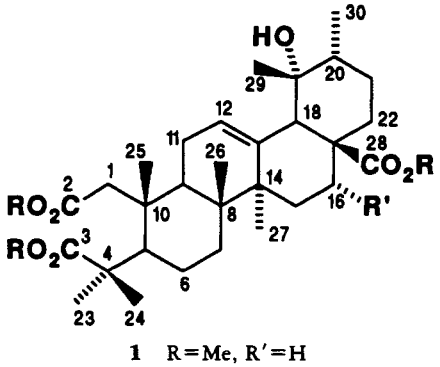
ABSTRACT.—A novel A-ring seco pentacyclic triterpene has been isolated from the stem bark of *Musanga cecropioides*, as well as the known oleanolic and ursolic acids, 2α-hydroxy oleanolic and ursolic acids, benthamic acid, 3-rhamnosyl benthamic acid, and tormentic acid, all as their methyl esters. The new triterpene has been characterized by spectroscopic methods as methyl 16α,19α-dihydroxy-2,3-secours-12-ene-2,3,28-trioate [**3**]. The isolation of the known compounds is also described.

In our continuing search for the chemical constituents of the family Cecropiaceae (2–4), we investigated chromatographically the polar material of *Musanga cecropioides* R. Brown (1), an endemic species widely used in African tropical medicine (5,6). In our previous work (7), we described the structure elucidation of the first A-ring seco pentacyclic triterpene, cecropiacic acid [**2**], as its methyl ester **1**, from the polar part of the extract of the stem bark of *M. cecropioides*. We now report the isolation of the second A-ring seco triterpene, musangic acid [**4**], from the same tissue, as its methyl ester **3**, as well as oleanolic and ursolic acids, 2α-hydroxy oleanolic and ursolic acids, tormentic acid, benthamic acid, and 3-rhamnosyl benthamic acid, as their methyl esters.

Exhaustive chromatography of the methylated EtOAc extract of the air-dried stem bark yielded, in addition to compound **1** (7) and the known triterpenes (8–10), a small amount of the new triterpenic constituent **3**.

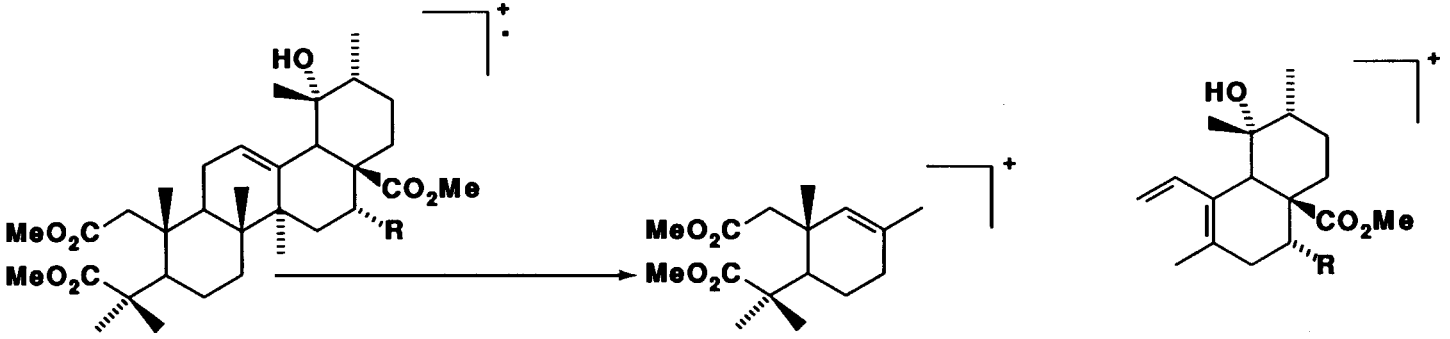
Compound **3** had the molecular composition $C_{33}H_{52}O_8$, and its spectral data were very similar to those of cecropiacic acid methyl ester [**1**] (7). Its ir spectrum showed absorptions for hydroxyls (3500 cm^{-1}), carbomethoxyls (1730 and 1700 cm^{-1}), olefin (1640), and *gem*-dimethyl (1360, 1380 cm^{-1}).

The ^{1}H-nmr spectrum of **3** was very similar to that of **1**: it showed resonances for six tertiary methyl groups at δ 0.67 (3H, 26-Me), 0.97 (3H, 29-Me), 1.16 (3H, 25-Me), 1.18 (3H, 23-Me), 1.20 (3H, 24-Me), 1.27 (3H, 27-Me), and a secondary methyl at δ 0.96 (3H, d, J = 6.8 Hz, 30-Me), a two-proton quartet at δ 2.29 (J = 18.6 Hz) attributed to the methylene protons at C-1, and a one-proton singlet at δ 2.51 assigned to the allylic proton at C-18. Furthermore, the singlet nature of the above hydrogen confirmed the presence of the C-19 hydroxyl function (11). The carbomethoxyl protons ap-



1 R=Me, R′=H
2 R=H, R′=H
3 R=Me, R′=OH
4 R=H, R′=OH

TABLE 1. Mass Spectral Fragmentation of **1** and **3**.



Compound	R	$[M]^+$	$[M-H_2O]^+$ $[M-2H_2O]^+$	$[a]^+$	$[b]^+$	$[b-H_2O]^+$ $[b-2H_2O]^+$	$[b-H_2O-HOAc]^+$	base peak
1	H	560[a]	542	282	278	260	200	43
3	OH	576	558	282	294	276	216	43
			540			258		

[a] *m/z*.

peared at δ 3.59 (C-3), 3.61 (C-2), and 3.63 (C-28). A one-proton triplet at ca. δ 4.00 was assigned to H-16β, and finally, a one-proton triplet at δ 5.31 (J = 3.5 Hz, H-12) confirmed the presence of the trisubstituted double bond.

Moreover, the mass spectrum of musangic acid methyl ester [**3**] (Table 1) exhibited important peaks at *m/z* 282 $[a]^+$ and 294 $[b]^+$, as a consequence of the retro-Diels-Alder fragmentation of the ring C (12). Also, the ions *m/z* 276 $[b-18]^+$, 234 $[b-H-CO_2Me]^+$, 217, 216, 195, 177, and 162 were quite diagnostic (12). It was evident from the fragments $[a]^+$ and $[b]^+$ that the two hydroxyl groups were located in rings D and E, as well as one of the three carbomethoxyl groups, as it is in cecropiacic acid methyl ester [**1**] (7). In addition, it is noteworthy that fragment $[a]^+$ has the same constitution as that of cecropiacic acid methyl ester [**1**]. As 30-Me appeared as a doublet, the second hydroxyl function should be secondary (13) and should be located at the C-16 position for the reasons that follow. It is known (13) that for triterpenoid skeletons, modifications in substitution pattern are always accompanied by systematic changes in the ^{1}H-nmr chemical shifts of the angular methyl groups, and that such changes are to a first approximation, additive. Also, it should be noted that substituents in rings D and E mainly affect 27-Me, 29-Me, and 30-Me. In effect, the chemical shift of 29-Me in musangic acid methyl ester [**3**] (0.96) is almost identical to that in **1** (0.97). On the other hand, the chemical shifts of 27-Me and 30-Me in **3** differ significantly from those in **1**. In fact, the latter methyls appeared at δ 1.20 and 0.91, respectively, in **1** and at δ 1.27 and 0.96 in **3**. The increment (ca. 0.06) observed in the second case is in agreement with the presence of an α-axial hydroxyl group at the C-16 position (13,14). In addition, in the work of Hart *et al.* (15), a C-22 proton geminal to a hydroxyl function appeared at δ 5.04. In **3**, however, the proton geminal to the secondary hydroxyl group resonated at ca. δ 4.00, which is far from the above value but almost identical to that found by Ito and Ogino (16) and Konoshima and Lee (17) for a 16β proton. Finally, the structure of methyl 16α,19α-dihydroxy-2,3-secours-12-ene-2,3,28-trioate was assigned to **3**. This structure was further confirmed by the ^{13}C-nmr spectrum (Table 2), the chemical shifts of which were assigned by means of the single-frequency off-resonance decoupling technique (18), by application of known chemical shift rules such as hydroxyl substitution (19) and from comparison of the spectrum of **1** treated in the previous study (7). The ^{13}C-nmr spectrum strongly supported the assigned structure **3** and confirmed the location of the different functional groups and, particularly, the stereochemistry of the C-16 hydroxyl function. In fact, the chemical shifts of the different carbons of the rings A (2,3-seco), B, C, and D in **3** were very comparable to the respective ones in **1**, confirming the identical environment in both cases. In addition, the ^{13}C-nmr spectrum of **3** displayed the signal for C-16 at considerably lower field (δ 73.4) than those observed values for related compounds with 16β-OH configuration (δ 64–67) (20,21). It is apparent that the α-axial orientation of the C-16 hydroxyl function is responsible for the downfield shift observed on the latter. This result is in conformity with the works of Doddrell *et al.* on similar derivatives (20). Compound **3** was, therefore, characterized as methyl 16α-19α-dihydroxy-2,3-secours-12-ene-2,3,28-trioate, and the natural product is 16α,19α-dihydroxy-2,3-secours-12-ene-2,3,28-trioic acid [**4**].

The polar material of *M. cecropioides* has been found to have a potent herbicidal activity (22). Both compounds **1** and **3** may be implicated in such activity. Studies leading to the confirmation of the said activity are now awaited.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H-nmr spectra were run on Perkin-Elmer R12 and R32 (90 MHz) and Brücker WP 200 SY (200.13 MHz) spectrometers, and ^{13}C-nmr spectrometer with a VFT-100 accessory. Mass spectra

TABLE 2. ^{13}C-nmr Spectra of Compounds **1** and **3** (50.32 MHz, $CDCl_3$, TMS as Internal Standard).

Carbon atom	Compound	
	1	**3**
1	41.4	41.5
2	171.9	171.9
3	179.8	179.8
4	46.1	46.1
5	47.8	48.7
6	20.9	20.8
7	31.9	31.8
8	41.7	41.7
9	38.9	38.8
10	39.8	39.9
11	23.8	18.5
12	129.0	129.3
13	138.0	137.5
14	41.68	42.4
15	28.2	31.8
16	25.5	73.4
17	47.8	53.8
18	53.2	53.3
19	73.0	72.0
20	41.1	38.8
21	25.9	23.9
22	37.3	33.4
23	24.0	24.0
24	16.1	15.8
25	16.5	16.3
26	18.8	18.8
27	27.4	27.2
28	178.3	177.2
29	27.7	27.78
30	23.7	23.7
3 × COOMe	50.8, 51.5, 51.7	50.8, 51.7, 52.0

were obtained with an LKB 9000S instrument, and ir spectra run as KBr discs on a Perkin-Elmer 727 B. Si gel GF_{254} (Merck) and Si gel 60 (70–230 mesh ASTM) (Merck) were used for tlc and cc, respectively, and the spots on tlc were visualized by spraying with a 50% aqueous solution of H_2SO_4 and heating at 150°.

PLANT MATERIAL.—The stem bark of *M. cecropioides* was collected at Mount Kala, Yaoundé area, Cameroon, on November 6, 1986, by Dr. G. Achoundong and one of the authors (D.L.). A voucher specimen is deposited in the Cameroon National Herbarium , Yaoundé.

EXTRACTION AND ISOLATION.—The pulverized, air-dried stem bark (5 kg) of *M. cecropioides* was macerated with MeOH (20 liters) at room temperature for 3 days and the solvent removed under reduced pressure to afford a gum (460 g) that was successively fractionated with hexane (20 liters) and EtOAc (20 liters). The EtOAc extract (38 g) was dissolved in the same solvent and treated with 2 N NaOH to give an organic fraction containing mainly nonsaponified triterpenic acids, an aqueous phase containing H_2O-soluble salts of the above acids, and a precipitate containing non-H_2O-soluble salts. The aqueous fraction and the precipitate were acidified with 2N HCl and extracted with EtOAc. After evaporation of the solvent, the extracts were treated in each case with an excess of an Et_2O solution of CH_2N_2 to give mixtures A (3.27 g) and B (5.7 g). The organic fraction was in turn concentrated under reduced pressure to furnish mixture C (13 g). Mixture A was chromatographed over Si gel (70 g) using hexane/EtOAc mixtures as eluent. Hexane-EtOAc (17:3) afforded **1** (100 mg) as colorless needles, mp 174–175° (hexane/EtOAc) and **3** (100 mg) as an amorphous powder. Repeated cc of mixture B on the same adsorbent (120 g) and elution with hexane-EtOAc (9:1) gave friedelin, mp 260–262°, and 3β-hydroxy friedelinol, mp 283°. Hexane-EtOAc (4:1) furnished a mixture of methyl 2α-hydroxyoleanolate and methyl ursolate, mp 221–223° (9), and hexane-EtOAc (7:3) eluted methyl tormentate, mp 157–160° (8). Mixture C, chromatographed over Si

gel (260 g) and eluted with hexane-EtOAc (7:3) gave 280 mg of a two-component powder (D). Hexane-EtOAc (1:4) eluted the fractions 52–54, subsequently methylated to give 0.5 g of mixture E. Mixture D, re-chromatographed over Si gel and eluted with hexane-$CHCl_3$ (1:4), afforded ursolic and oleanolic acids (mixture, one spot on tlc) and benthamic acid (10), subsequently methylated (20 mg), mp 138°. Mixture E was re-chromatographed on the same adsorbent, and elution with petroleum ether-EtOAc (3:2) afforded the methylbenthamate 3-rhamnosyl (30 mg), mp 175–177°.

METHYL 19α-HYDROXY-2,3-SECOURS-12-ENE-2,3,28-TRIOATE [**1**].—Mp 174–175° (hexane/EtOAc), ir ν max 3575, 3530 (sh, OH), 1740, 1720, (esters) 1630 (trisubstituted double bond), 1450, 1430 (C-Me), 1390, 1370 (*gem*-dimethyl), 1340, 1310, 1250, 1230, 1220 (C-O), 1190, 1140, 1100, 1090, 1070, 1040, 1030, 1000, 970, 930, 890, 875, 860, 840, 820, 800, 760, 750; ^{1}H nmr (δ, $CDCl_3$, 200.13 MHz) 0.66 (3H, s, 26-Me), 0.91 (3H, d, J = 6.5 Hz, 30-Me), 0.96 (3H, s, 29-Me), 1.17 (3H, s, 25-Me), 1.20 (3H, s, 27-Me), 1.22 (3H, s, 23-Me), 1.25 (3H, s, 24-Me), 3.57 (3H, s, 3-CO_2Me), 3.58 (3H, s, 2-CO_2Me), 3.60 (3H, s, 28-CO_2Me), 5.32 (1H, t, J = 3.6 Hz, H-12), 2.55 (1H, s, H-18), 2.28 (2H, d, J = 18.2 Hz, H_2-1); eims (70 eV) *m/z* $[M]^+$ 560, $[M-H_2O]^+$ 542, $[M-MeOH]^+$ 528, 510, $[RDA]^+$ 282, $[M-RDA]^+$ 278, 275, $[M-RDA-H_2O]^+$ 260, 205, 204, 201, 179, 146, 133, 131, 121, 43 (100%).

METHYL 16α,19α-DIHYDROXY-2,3-SECOURS-12-ENE-2,3,28-TRIOATE [**3**].—Amorphous powder, ir ν max 3500 (br, OH), 1730, 1700 (esters), 1640 (trisubstituted double bond), 1580, 1440, 1420 (C-Me), 1360, 1380 (*gem*-dimethyl), 1340, 1250–1200 (C-O), 1180, 1110, 1070, 1000, 975, 950, 920, 900, 870, 840, 830, 795, 960, 720, 630; ^{1}H nmr (δ, $CDCl_3$, 200.13 MHz) see Discussion section; eims (70 eV) *m/z* $[M]^+$ 576, $[M-H_2O]^+$ 558, $[M-2H_2O]^+$ 540, $[RDA]^+$ 294, $[M-RDA]^+$ 282, $[M-RDA-18]^+$ 276, 258, $[RDA-H-CO_2Me]^+$ 234, 217, 216, 205, 195, 179, 177, 165, 162, 159, 133, 131, 121, 105, 43 (100%); ^{13}C nmr see Table 2. *Anal.* calcd for $C_{33}H_{52}O_8$: C 68.71, H 9.08%; found C 68.91, H 8.97%.

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LITERATURE CITED

1. C. Berg, *Taxon*, **27**, 39 (1978).
2. F.N. Ngounou, D. Lontsi, J.F. Ayafor, and B.L. Sondengam, *Phytochemistry*, **26**, 3080 (1987).
3. F.N. Ngounou, D. Lontsi, and B.L. Sondengam, *Phytochemistry*, **27**, 301 (1988).
4. F.N. Ngounou, D. Lontsi, and B.L. Sondengam, *Phytochemistry*, **27** 2287 (1988).
5. A. Bouquet, "Féticheurs et médecines traditionnelles du Congo," ORSTOM, Brazzaville, 1969.
6. F.R. Irvine, "Woody Plants of Ghana," Oxford University Press, London, 1961, pp. 446–447.
7. D. Lontsi, B.L. Sondengam, J.F. Ayafor, and J.D. Connolly, *Tetrahedron Lett.*, **28**, 6683 (1987).
8. C.M. Ojinnaka, J.I. Okogun, and D.A. Okorie, *Phytochemistry*, **19**, 2482 (1980).
9. A.T. Glen, W. Lawrie, and M. El Garby Younes, *J. Chem. Soc. C*, 510 (1980).
10. J. Barmejio, J.L. Breton, G. de la Fuente, and A.G. Gonzales, *Tetrahedron Lett.*, **47**, 4647 (1967).
11. P. Potier, B.C. Das, A. Bui, M. Janot, and A.H. Pourrat, *Bull. Soc. Chim. Fr.*, 3458 (1966).
12. C. Djerassi, H. Budzikiewicz, and J.M. Wilson, *Tetrahedron Lett.*, **7**, 263 (1962).
13. H.T. Cheung and D.G. Williamson, *Tetrahedron*, **25**, 119 (1969).
14. R. Savoir, R. Ottinger, B. Turch, and G. Chiurdoglu, *Bull. Soc. Chim. Belg.*, **76**, 371 (1967).
15. N.K. Hart, J.A. Lamberton, and A.A. Sioumis, *Aust. J. Chem.*, **29**, 655 (1976).
16. S. Ito and T. Ogino, *Tetrahedron Lett.*, 1127 (1967).
17. T. Konoshima and K.H. Lee, *J. Nat. Prod.*, **49**, 650 (1986).
18. F.W. Wehrli and T. Wirtlin, "Interpretation and Carbon-13 NMR Spectra," Heydin and Sons, London, 1980, pp. 64–83.
19. S. Seo, Y. Tomita, and K. Tori, *Tetrahedron Lett.*, 7 (1975).
20. D.M. Doddrell, P.W. Kong, and K.G. Lewis, *Tetrahedron Lett.*, 2381 (1974).
21. H. Ishii, S. Seo, K. Tori, T. Tozyo, and Y. Yoshimura, *Tetrahedron Lett.*, 1227 (1977).
22. H. Ohigashi, M. Kaji, J. Hoshino, J. Jato, and K. Koshimizu, *Japanese Journal of African Studies*, **3**, 30 (1987).

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