Pentacyclic Triterpenes and Other Constituents from *Ficus cordata* (Moraceae)

Herve M. P. Poumale[a,b], Rodrigue T. Kengap[a], Jean Claude Tchouankeu[a], Felix Keumedjio[a], Hartmut Laatsch[b], and Bonaventure T. Ngadjui[a]

[a] Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, PO Box 812, Yaoundé, Cameroon
[b] Department of Organic and Biomolecular Chemistry, University of Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany

Reprint requests to Dr. Herve M. P. Poumale. Fax: +237 2222 1873. E-mail: poumale@yahoo.fr

Z. Naturforsch. **2008**, *63b,* 1335 – 1338; received May 18, 2008

Two new pentacyclic triterpenes 8,26-cyclo-urs-21-en-3β,20β-diol (**1**) and 3β-acetoxy-8,26-cyclo-ursan-20β-ol (**2**) together with 3-friedelanone, oleanolic acid, betulinic acid, lupeol acetate, α- and β-amyrine, 3,5,7,4′-tetrahydroxyflavane, and 3,5,7,3′,4′-pentahydroxyflavane were isolated from the stem bark of *Ficus cordata* (Moraceae). The structures of these secondary metabolites were established using 1D and 2D NMR spectra and by comparison with published data or with authentic samples. Compounds **1** and **2** exhibited weak antibacterial and no antifungal activity.

Key words: *Ficus cordata*, Moraceae, 8,26-Cyclo-urs-21-en-3β,20β-diol, 3β-Acetoxy-8,26-cyclo-ursan-20β-ol, Antibacterial

Introduction

Ficus cordata Thunb. (Moraceae) is a savanna tree of around ten meters height present in Senegal, Angola, South Africa and Cameroon [1, 2]. The leaves of this plant are used against hyperaesthesia, ataxia, muscle tremor and padding motions and can kill heifers 48 h after ingestion [3]. *Ficus septica* is known for its purgative and emetic effects [4]. Previous phytochemical studies of the genus *Ficus* resulted in the isolation of flavonoids, coumarins, alkaloids, steroids, ceramides and triterpenes [5]. In the search for the chemical constituents of Cameroonian medicinal plants [6], we have examined the crude dichloromethane/methanol (1 : 1) extract of *Ficus cordata*. In this paper we describe the isolation and characterization of two new pentacyclic triterpenes: 8,26-cyclo-urs-21-en-3β,20β-diol (**1**) and 3β-acetoxy-8,26-cyclo-ursan-20β-ol (**2**). To the best of our knowledge, no previous phytochemical study has been reported on *Ficus cordata* Thunb.

Results and Discussion

The stem bark of *Ficus cordata* was extracted with dichloromethane/methanol (1 : 1) for 24 h. The extract was submitted to flash chromatography, repeated column chromatography and preparative TLC to afford 3-friedelanone, oleanolic acid, betulinic acid, lupeol acetate, α- and β-amyrine, 3,5,7,4′-tetrahydroxyflavane, 3,5,7,3′,4′-pentahydroxyflavane, and two new pentacyclic triterpenes (**1** and **2**). The ^{1}H and ^{13}C NMR and MS data of the known compounds were consistent with those reported in the literature.

Compound **1** gave a positive Liebermann Buchard test characteristic of triterpenoids and was obtained as an amorphous solid from hexane-ethyl acetate (3 : 1). Its (+)-ESI HR mass spectrum exhibited a *pseudo*molecular ion peak at *m/z* = 441.37257 $[M+H]^+$, corresponding to a molecular formula $C_{30}H_{49}O_2$. The IR spectrum showed a broad signal at ν_{max} = 3420 cm^{-1} corresponding to free hydroxyl groups. The absorption bands at ν_{max} = 2934 and 886 cm^{-1} indicated a cyclopropane ring while that at 1340 cm^{-1} showed the presence of C=C [7]. The ^{13}C NMR and DEPT spectra displayed signals for seven methyl, nine methylene and seven methine groups as well as seven quaternary carbon atoms; two sp^3 carbons assignable to C-3 and C-20 at δ = 79.2 (CH) and 81.6 (C_q) were oxygenated; carbonyl groups were absent.

The ^{1}H NMR spectrum of compound **1** confirmed the seven methyl groups by signals between δ = 0.78

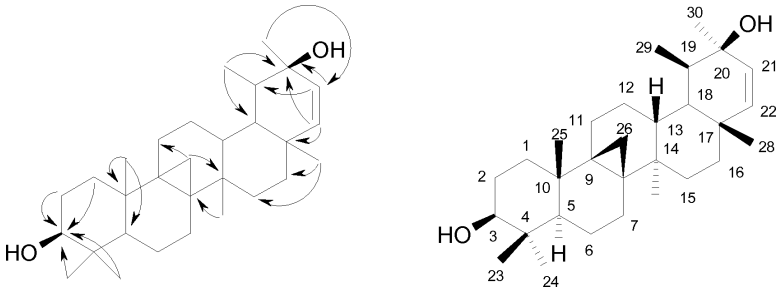

Fig. 1. Selected HMBC correlations of 8,26-cyclo-urs-21-en-3β,20β-diol (**1**).

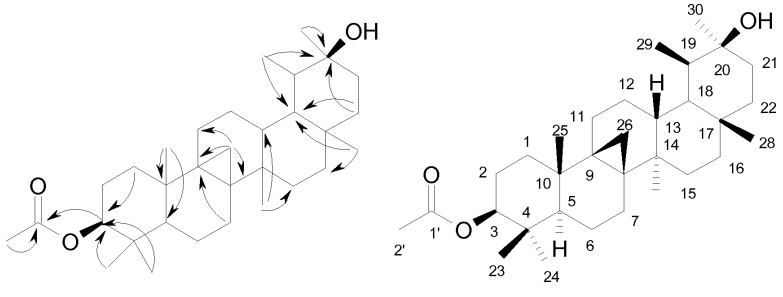

Fig. 2. Selected HMBC correlations of 3β-acetoxy-8,26-cyclo-ursan-20β-ol (**2**).

and 1.26, of which six were singlets and one was a doublet (δ = 0.88, J = 6.4 Hz). An AB signal at δ = 0.36 and 0.56 (J = 4.1 Hz) was characteristic for a methylene group of a tetrasubstituted cyclopropyl moiety [8]. The ^{1}H NMR spectrum showed also a disubstituted *cis* double bond at δ = 5.59 (br d, 2H). The corresponding two olefinic carbon atoms at δ = 137.0 and 128.8 are characteristic for C-21 and C-22 of urs-21-ene derivatives [9].

From the molecular formula $C_{30}H_{48}O_2$, seven double bond equivalents are deduced. As two of them are due to the double bond and the cyclopropane ring, compound **1** must be a pentacyclic triterpene, probably of the ursane type [10]. The broad doublet at δ = 3.42 (J = 12.0 Hz) was consistent with the presence of a β-hydroxyl group at C-3. Due to the correlation between H-28 and H-29 (and not H-30) shown in the NOESY spectrum, the relative stereochemistry of the second -OH group at position C-20 was determined to be β. The NOESY correlation between H-25, H-26 and H-23 was also observed. The HMBC correlations (Fig. 1) for H-21, H-22/C-17 and C-20; H-30/C-19, C-20 and C-21; H-26/C-8, C-9, C-11, and C-14 as well as for H-23, H-24/C-3, C-4 and C-5 confirmed the assignments of major proton and carbon resonances in **1** together with the locations of the cyclopropyl and hydroxyl groups. From the foregoing data compound **1** was characterized as 8,26-cyclo-urs-21-en-3β,20β-diol, which is described here for the first time.

Compound **2** was isolated as an amorphous solid from the same fraction B eluted with hexane/ethyl acetate (3 : 1). The EI mass spectrum exhibited a molecular ion peak at m/z = 484 corresponding to the molecular formula $C_{32}H_{52}O_3$. The IR spectrum showed a broad signal at ν_{max} = 3409 cm^{-1} indicating free hydroxyl groups, and ester signals at ν_{max} = 1733 (C=O) and 1246 (C–O) cm^{-1}. The absorption bands at ν_{max} = 2939 and 886 cm^{-1} indicated the presence of a cyclopropane ring.

Comparison of the ^{1}H and ^{13}C NMR spectra (Experimental Section) of **1** and **2** showed strong similarities of both compounds. As in **1**, the presence of an AB signal at δ_H = 0.54 and 0.32 (J = 4.2 Hz, δ_C = 29.7) confirmed the CH_2 group of a cyclopropyl moiety; two signals of oxygenated sp^3 carbon atoms were also observed at δ = 80.6 (C-3) and 76.6 (C-20). The major difference between the NMR spectra of both compounds was the appearance of an acetyl signal at δ_H = 2.03 (s), δ_C = 21.3 (CH_3) and 171.0 (C_q) and the absence of double bond signals. Instead, two further methylene signals were visible in the spectrum of **2**. The down-field shift of H-3 from δ = 3.42 in **1** to δ = 4.55 (dd, J = 5.6 and 10.9 Hz) in **2** suggested that the acetoxy group should be located in position 3. The analysis of the data and the comparison with those of compound **1** and the literature [11] indicated that compound **2** is 3β-acetoxy-8,26-cyclo-ursan-20β-ol, which is described here for

the first time. This structure is supported by HMBC data (Fig. 2).

The antifungal and antibacterial activities of compounds **1** and **2** were determined using the agar diffusion method with 9 mm paper disks loaded with 40 μg/mL of each compound. 8,26-Cyclo-urs-21-en-3β,20β-diol (**1**) showed weak activities against *Bacillus subtilis* (14 mm inhibition zone diameter), *Streptomyces viridochromogenes* (Tü 57) (12 mm), *Mucor miehei* (13 mm), *Chlorella vulgaris* (11 mm) and *Scenedesmus subspicatus* (10 mm); 3β-acetoxy-8,26-cyclo-ursan-20β-ol (**2**) showed weak activities only against *Bacillus subtilis* (11 mm) and *Escherichia coli* (14 mm).

Experimental Section

Materials and methods

NMR spectra were measured on Varian Unity 300 (300.145 MHz) and Varian Inova 500 (499.876 MHz) spectrometers. ESI MS was recorded on a Finnigan LCQ with a quaternary pump Rheos 4000 (Flux Instrument). ESI HR mass spectra were recorded on a Bruker FTICR 4.7 T mass spectrometer. EI MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosene as reference substance for HREI MS. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer from films. Flash chromatography was carried out on silica gel (230 – 400 mesh). R_f values were measured on Polygram SIL G/UV254 (Macherey-Nagel & Co.).

Plant material

The stem bark of *Ficus cordata* was collected in March 2006, from "Mont Kala", in the Center Province of Cameroon, and the plant was identified by Mr. V. Nana of the National Herbarium of Yaounde. A specimen has been deposited in the National Herbarium, Yaounde, Cameroon (Ref. Nr. 8613).

Extraction and isolation

The powdered stem bark of *Ficus cordata* (1.8 kg) was extracted with CH_2Cl_2/MeOH (1 : 1) at r. t. for 24 h. After removing the solvents by evaporation under reduced pressure, the crude extract (155 g) was chromatographed on silica gel. Elution with the binary solvent systems hexane/ethyl acetate (1 : 1), ethyl acetate and methanol, afforded three fractions A–C.

Fraction A (17.0 g) was chromatographed on silica gel and eluted with a mixture of hexane/ethyl acetate of increasing polarity to yield α- and β-amyrine (35.0 mg and 97 mg, respectively) [12], betulinic acid (29.1 mg) [13], and lupeol acetate (8.0 mg) [14].

Fraction B (12.0 g) was chromatographed on silica gel and eluted using hexane/ethyl acetate (3 : 1) to deliver 8,26-cyclo-urs-21-en-3β,20β-diol (7.7 mg) (**1**), 3β-acetoxy-8,26-cyclo-ursan-20β-ol (3.5 mg) (**2**) and 3-friedelanone (3.3 mg) [15].

Fraction C (36.0 g), chromatographed on silica gel and eluted with a mixture of CH_2Cl_2/MeOH of increasing polarity, produced in the same way oleanolic acid (11.3 mg) [16], betulinic acid (7.9 mg), 3,5,7,4′-tetrahydroxyflavane (60.1 mg) [17] and 3,5,7,3′,4′-pentahydroxyflavane (57.0 mg) [18].

*8,26-Cyclo-urs-21-en-3β,20β-diol (**1**)*

Amorphous solid. – R_f = 0.52 (CH_2Cl_2). – $[\alpha]_D^{25}$ = +37° (c = 0.07, [D$_6$]acetone). – IR (film): ν = 3420, 3415, 2934, 2869, 1458, 1377, 1340, 1268, 1188, 1149, 1099, 1025, 1006, 993, 971, 916, 886, 737 cm^{-1}. – ^{1}H NMR (300 MHz, $CDCl_3$, 35 °C, TMS): δ = 0.36 (1H, d, J = 4.1 Hz, H-26), 0.56 (1H, d, J = 4.1 Hz, H-26), 0.78 (3H, s, H-28), 0.80 (3H, s, H-24), 0.88 (3H, d, J = 6.4 Hz, H-29), 0.91 (3H, s, H-27), 0.94 (3H, s, H-23), 1.00 (3H, s, H-25), 1.26 (3H, s, H-30), overlapping multiplets 2.22 – 1.10 (19H, m, H-1, 2, 5, 6, 7, 11, 12, 13, 15, 16, 18), 3.20 (2H, br s, disappeared with D_2O -OH), 3.42 (1H, br d, J = 12.0 Hz, H-3), 5.59 (2H, br d, J = 1.8 Hz, H-21, H-22). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.8 (q, C-23), 18.7 (q, C-29), 18.9 (t, C-7), 20.0 (t, C-1), 20.7 (t, C-15), 21.8 (q, C-27), 24.9 (q, C-25), 25.0 (q, C-24), 26.0 (t, C-6), 27.0 (t, C-12), 27.1 (q, C-28), 28.4 (s, C-17), 29.0 (q, C-30), 30.8 (s, C-8), 31.1 (s, C-9), 32.6 (t, C-2), 33.7 (t, C-11), 36.2 (s, C-14), 37.0 (t, C-16), 40.1 (s, C-4), 41.3 (t, C-26), 46.0 (s, C-10), 48.1 (d, C-13), 49.0 (d, C-18), 49.5 (d, C-19), 52.8 (d, C-5), 79.2 (d, C-3), 81.6 (s, C-20), 128.8 (d, C-21), 137.0 (d, C-22). – MS (EI, 70 eV): *m/z* (%) = 440 (38) [M]$^+$, 424 (88), 409 (66), 392 (20), 381 (40), 365 (13), 342 (21), 325 (33), 297 (47), 269 (17), 255 (41), 215 (25), 203 (77), 175 (90), 147 (93), 121 (94), 107 (100), 95 (96), 81 (77), 55 (52), 43 (33). – HRMS ((+)-ESI): *m/z* = 441.37257 (calcd. 441.37264 for $C_{30}H_{49}O_2$, [M+H]$^+$).

*3β-Acetoxy-8,26-cyclo-ursan-20β-ol (**2**)*

Amorphous solid. – R_f = 0.64 (CH_2Cl_2). – $[\alpha]_D^{25}$ = +34° (c = 0.1, [D$_6$]acetone). – IR (film): ν = 3409, 2939, 2870, 1733, 1541, 1458, 1373, 1246, 1172, 1135, 1094, 1027, 979, 922, 886 cm^{-1}. – ^{1}H NMR (300 MHz, $CDCl_3$, 35 °C, TMS): δ = 0.32 (1H, d, J = 4.2 Hz, H-26), 0.54 (1H, d, J = 4.1 Hz, H-26), 0.82 (3H, s, H-28), 0.86 (3H, br d, H-29), 0.86 (9H, s, H-24, H-25, H-27), 0.93 (3H, s, H-23), 1.23 (3H, s, H-30), overlapping multiplets 2.10 – 1.10 (23H, m, H-1, 2, 5, 6, 7, 11, 12, 13, 15, 16, 18, 21, 22), 2.03 (3H, s, H-2′), 4.55 (1H, dd, J = 5.6, 10.9 Hz, H-3). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 15.1 (q, C-29), 17.9 (q, C-27), 18.0 (q, C-25), 19.3 (q,

C-24), 20.0 (t, C-6), 20.9 (t, C-2), 21.3 (q, C-2′), 22.7 (t, C-22), 25.4 (t, C-21), 25.8 (q, C-23), 25.9 (q, C-30), 26.4 (t, C-1), 26.8 (s, C-9), 28.0 (s, C-8), 29.6 (q, C-28), 29.7 (t, C-15), 29.7 (t, C-26), 31.0 (t, C-12), 31.6 (t, C-11), 32.8 (t, C-7), 35.4 (t, C-16), 35.6 (s, C-17), 39.4 (d, C-13), 45.3 (s, C-4), 47.1 (d, C-19), 47.8 (s, C-10), 48.8 (s, C-14), 52.0 (d, C-18), 52.1 (d, C-5), 76.6 (s, C-20), 80.6 (d, C-3), 171.0 (s, C=O). – MS (EI, 70 eV): *m/z* (%) = 484 (13) $[M]^+$, 466 (11), 424 (8), 398 (10), 383 (12), 297 (17), 227 (16), 203 (14), 175 (22), 135 (19), 121 (22), 107 (25), 95 (30), 69 (23), 55 (26), 43 (100). – HRMS ((+)-ESI): *m/z* = 485.42513 (calcd. 484.42519 for $C_{32}H_{53}O_3$, $[M+H]^+$).

Antimicrobial assay

Agar diffusion tests were performed in the usual manner [19] with *Bacillus subtilis* and *Escherichia coli* (on peptone agar), *Staphylococcus aureus* (Bacto nutrient broth), *Streptomyces viridochromogenes* (M test agar), the fungi *Mucor miehei* and *Candida albicans* (Sabouraud agar), and three microalgae (*Chlorella vulgaris, Chlorella sorokiniana* and *Scenedesmus subspicatus*).

The test substances were dissolved in chloroform/methanol (87 : 13) azeotrope. Paper disks (∅ 9 mm) were impregnated each with 40 μg of the substance using a 100 μL syringe, dried for 1 h under sterile conditions and placed on the pre-made agar test plates. Plates with bacteria and fungi were kept in an incubator at 37 °C for 12 h, micro algae plates were kept for three days at r. t. in a day light incubator. The diameter of inhibition zones was measured.

Acknowledgements

We thank R. Machinek for the NMR spectra, H. Frauendorf for the mass spectra, and F. Lissy for microbiological work. H. M. P. P. is thankful to the DAAD (Deutscher Akademischer Austauschdienst) for a research grant, and B. T. N. is grateful to DAAD for a one month research visit to Chemistry Department at the University of Göttingen.

[1] J. Berhaut, *Flore Illustrée du Sénégal, Dicotyledones, Tome IV, Linacees a Nympheacees*, Gouvernement du Sénégal, Ministère du Développement Rural et de l'Hydraulique Direction des Eaux et Forets, Dakar, **1979**, pp. 429 – 482.

[2] B. Sabatie, *Flore du Cameroun, Moraceae (incl. Cecropiaceae)*, Ministère de l'Enseignement Supérieur et de la Recherche Scientifique (MESRES) (Ed.), Yaoundé-Cameroun, **1985**, pp. 154 – 155.

[3] J. G. Myburgh, N. Fourie, J. J. Van der Lugt, T. S. Kellerman, S. T. Cornelius, C. A. Ward, *Onderstepoort J. Vet. Res*. **1994**, *61*, 171 – 176.

[4] J. C. Liao, in *Flora of Taiwan*, Vol. 2, (2nd ed.), Editorial Committee of the Flora of Taiwan, Taipei, **1996**, pp. 177 – 180.

[5] C. C. F. Simo, S. F. Kouam, H. M. P. Poumale, K. I. Simo, B. T. Ngadjui, R. I. Green, K. Krohn, *Biochemical Systematics and Ecology* **2008**, *36*, 238 – 243.

[6] B. T. Ngadjui, B. M. Abegaz, in *Studies in Natural Products Chemistry, Bioactive Natural Products*, Vol. 29 (Ed.: Atta-Ur-Rahman), Elsevier, Oxford, **2003**, pp. 761 – 805.

[7] S. Hayat, Atta-ur-Rahman, M. I. Choudhary, K. M. Khan, H. Latif, E. Bayer, *Z. Naturforsch*. **2002**, *57b*, 233 – 238.

[8] H. T. Cheung, D. G. Williamson, *Tetrahedron* **1969**, *25*, 119 – 128.

[9] F. Liwei, S. Zhang, W. N. L. Jinlan, M. Zhao, J. Sakai, H. Toshiaki, M. Tomokazu, T. Kataoka, S. Oka, K. Miwa, H. Katutoshi, A. Masayoshi, *J. Nat. Prod*. **2005**, *68*, 198 – 206.

[10] K. Hisashi, O. Haruo, *Phytochemistry* **1989**, *28*, 1703 – 1710.

[11] A. K. Chakravarty, B. Das, T. Sarkar, K. Masuda, Y. Arai, K. Shiojima, *Tetrahedron* **1998**, *54*, 6065 – 6078.

[12] M. Shibuya, T. Xiang, Y. Katsube, M. Otsuka, H. Zhang, Y. Ebizuka, *J. Am. Chem. Soc*. **2007**, *129*, 1450 – 1455.

[13] R. Csuk, K. Schmuck, R. Schaefer, *Tetrahedron Lett*. **2006**, *47*, 8769 – 8770.

[14] I. Chatterjee, A. K. Chakravarty, A. Gomes, R. Daboia, K. V. Naja, *Journal of Ethnopharmacology* **2006**, *106*, 38 – 43.

[15] P. Tane, A. Tsopmo, D. Ngnokam, F. Ayafor, *Tetrahedron* **1996**, *52*, 14989 – 14994.

[16] B. M. Fraga, C. E. Diaz, N. Quintana, *J. Nat. Prod*. **2006**, *69*, 1092 – 1094.

[17] J. Larsson, J. Gottfries, L. Bohlin, A. Backlund, *J. Nat. Prod*. **2005**, *68*, 985 – 991.

[18] A. Seyoum, K. Asres, F. K. El-Fiky, *Phytochemistry* **2006**, *67*, 2058 – 2070.

[19] R. P. Maskey, R. N. Asolkar, E. Kapaum, I. Wagner-Döbler, H. Laatsch, *J. Antibiot*. **2002**, *55*, 643 – 649.