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TRITERPENES FROM *PRUNUS AFRICANA* BARK

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Key Word Index—*Prunus africana*; Rosaceae; triterpenic acids; 24-*O*-*trans*-ferulyl-2α,3α-dihydroxy-urs-12en-28-oic acid.

Abstract—A chloroformic extract of barks of *Prunus africana* was found to contain triterpenic acids including derivatives of ursolic and oleanolic acids. Among them 24-*O*-*trans*-ferulyl-2α,3α-dihydroxy-urs-12-en-28-oic acid, an original compound, was isolated. Their structure was established by chemical and spectral analysis.

INTRODUCTION

Prunus africana (Kalk.), also known as *Pygeum africanum* (Hook.), is a tree widely distributed in mountain forests of Central Africa. Its barks are used for the treatment of benign prostatic hypertrophy [1]. In continuation of studies on an extract of bark, known triterpenic acids, 2α,3α-dihydroxyurs-12-en-28-oic acid (**1**), 2α,3β-dihydroxyurs-12-en-28-oic acid (**2**), 2α,3β-dihydroxyolean-12-en-28-oic acid (**3**), 3β,24-dihydroxyurs-12-en-28-oic acid (**4**), 2α,3α,23-trihydroxyurs-12-en-28-oic acid (**5**), 2α.3α,24-trihydroxyurs-12-en-28-oic acid (**6**), 24-*O*-*trans*-ferulyl-3β-hydroxy-urs-12-en-28-oic acid (**7**), 24-*O*-*cis*-feruly-3β-hydroxy-urs-12-en-28-oic acid (**8**), have been isolated and characterized from this plant for the first time. In addition, to the above 24-*O*-*trans*-ferulyl-2α,3α-dihydroxy-urs-12-en-28-oic acid (**9**), a new triterpenic acid, has been also isolated. The structures of these compounds were elucidated by spectral analysis after acetylation and/or methylation. The ^{1}H and ^{13}C NMR assignments were performed in comparison with the published spectral data.

RESULTS AND DISCUSSION

The chloroform extract of the barks of *Prunus africana* is a complex mixture which contains, among other products, a large amount of triterpenic acids. Their presence has been characterized by GC-mass spectrometry of the crude extract after trimethylsilylation. The mass spectra present ions at *m/z* 320 and *m/z* 279 resulting from the retro-Diels–Alder fragmentation characteristic of the ursane and oleane skeleton. Furthermore they possess an ion at *m/z* 203 characteristic of Δ^{12}-triterpenoids [2].

The extract was subjected to several separation techniques (Sephadex®, silica gel column, semipreparative HPLC) in order to isolate the triterpenic compounds. Five fractions (A, B, C, D and E) were obtained. Fraction A treated with diazomethane and subjected to chromatography led to isolation of methyl 2α,3α-dihydroxyurs-12-en-28-oate (**1a**). Fractions B, C and D were methylated and acetylated then chromatographed to yield methyl 2α,3β-diacetoxyolean-12-en-28-oate (**3a**) and methyl 3β,24-diacetoxyurs-12-en-28-oate (**4a**) from fraction B, methyl 2α,3β-diacetoxyurs-12-en-28-oate (**2a**) and methyl 2α,3α,23-triacetoxyurs-12-en-28-oate (**5a**) from fraction C and methyl 2α,3α,24-triacetoxyurs-12-en-28-oate (**6a**) from fraction D. Compounds **7**, **8** and **9** were isolated by silica gel column or semipreparative HPLC from Fraction E. Identification of the known compounds (**1a–6a**) was based on comparison of their spectral properties with those reported in the literature. In the ^{1}H NMR spectra of these compounds, the signal of H-18 permitted the distinction between the oleane and ursane skeleton [3]. The chemical shifts of C-12 and C-13 (δ 125 and 138 in ursane, δ 122 and 144 in oleane) and H-12 (δ 5.20–5.25) suggests that these compounds are Δ^{12}-unsaturated triterpenes [4]. The stereochemistry of the hydroxyl or acetoxyl at C-2 and C-3 is clearly established by characteristic data from the ^{1}H NMR and ^{13}C NMR spectra [4]. In the ^{1}H NMR spectra of **4a**, **5a** and **6a**, a greater difference is observed between the chemical shifts of the protons H-23 than of the protons H-24. A study of the differences ($\Delta\delta_{2-3}$) between the chemical shifts of H-2 and H-3 corroborated these assignments [5].

The structure of **7** was elucidated by the NMR spectrum and comparison with published spectral data [6]. The triterpenic acid is linked by an ester function

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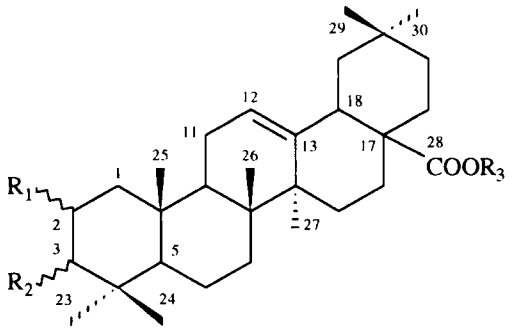
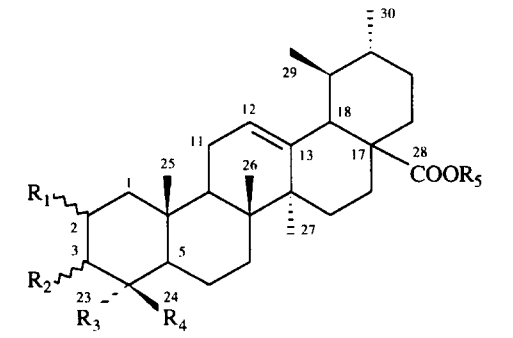
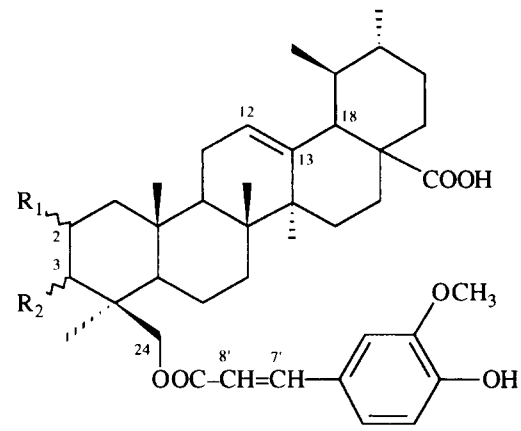


	R_1	R_2	R_3
3	αOH	βOH	H
3a	αOAc	βOAc	Me

involving the C-24 hydroxyl, the two protons at C-24 are very deshielded with regard to H-3 (δ 4.47 and 4.27 versus 3.32). Basic hydrolysis of **7** with 10% aqueous NaOH. gives the two constitutive fragments of the molecule, *trans*-ferulic acid and 3β,24-dihydroxyurs-12-en-28-oic acid (**4**). Compound **8**, 24-*O*-*cis*-ferulyl-3β-hydroxy-urs-12-en-28-oic acid, is mixed with **7**. In the ^{1}H NMR spectra of the mixture the differences between **7** and its isomer **8** appear clear, especially the chemical shifts of the feruloyl moiety protons. Indeed, the coupling constants of the conjugated olefinic protons allow the establishment of the *cis* configuration



	R_1	R_2	R_3	R_4	R_5
1	αOH	αOH	Me	Me	H
1a	αOH	αOH	Me	Me	Me
2	αOH	βOH	Me	Me	H
2a	αOAc	βOAc	Me	Me	Me
4	H	βOH	Me	CH_2OH	H
4a	H	βOAc	Me	CH_2OAc	Me
5	αOH	αOH	CH_2OH	Me	H
5a	αOAc	αOAc	CH_2OAc	Me	Me
6	αOH	αOH	Me	CH_2OH	H
6a	αOAc	αOAc	Me	CH_2OAc	Me



	R_1	R_2	$C_{7'}$=$C_{8'}$
7	H	βOH	*trans*
8	H	βOH	*cis*
9	αOH	αOH	*trans*

(J = 13 Hz) and *trans* configuration (J = 16 Hz). Compounds **7** and **8** were previously isolated from *Stizophyllum riparium* (Bignoniaceae) [6].

Compound **9**, 24 - *O* - *trans* - feruloyl - 2α,3α - dihydroxy - urs - 12 - en - 28 - oic acid, is a new triterpenic acid. In the CI-mass spectrum, the $[M]^+$ appears at m/z 664.9 (calculated for $C_{40}H_{56}O_8$), together with m/z 648 $[M-OH]^+$, 620 $[M-COOH]^+$ and 603 $[M-COOH\text{-}OH]^+$. The EI-mass spectrum shows characteristic fragmentations of ferulic acid and the Δ^{12}-triterpene skeleton (m/z 248 and 203) [2, 7], and by cleavage of the ester bond, the ions corresponding to triterpenic acid and phenolic acid moieties (m/z 471 and 178, respectively). Furthermore, basic hydrolysis of **9** with 10% aqueous NaOH yields 2α,3α,24-trihydroxyurs-12-en-28-oic acid (**6**) and *trans*-ferulic acid, both identified by GC and ^{1}H NMR by comparison with original samples. The comparison of ^{1}H NMR spectra of **6**, **6a** and **9** shows that bond between **6** and *trans*-ferulic acid involves the hydroxyl at C-24, as for **7** and **8** (Table 1). Indeed, esterification of the hydroxyl deshields the gemina protons. In the ^{1}H NMR spectra of **6** and **9**, only the protons H-24$_a$ and H-24$_b$ are significantly deshielded (respectively δ 3.8 and 4.1 versus δ 4.3 and 4.7). Conversely, esterification of the C-2 and C-3 hydroxyl would deshield protons H-2 and H-3 as for **6a** which is an acetoxylated compound (respectively δ 5.45 and 5.35 for H-2 and H-3).

Table 1. ^{1}H NMR spectral data of compounds **6**, **6a** and **9** (δ ppm, pyridine-d_5)

	6	**6a**	**9**
H-2	4.50	5.45	4.50
H-3	4.60	5.35	4.75
H-24$_A$	3.80	4.15	4.40
H-24$_B$	4.10	4.50	4.70

EXPERIMENTAL

General experimental procedures. The NMR spectra were recorded in $CDCl_3$ or C_5D_5N at 200 and 50 MHz for the ^1H and ^{13}C NMR, respectively, and 400 MHz for the correlated spectra. GC sepns were carried out with detection by FID. The column, used with N_2 (0.7 ml min^{-1}) as carrier gas, was a HP-1 capillary column (cross-linked methyl silicone gum phase, length 25 m, id 0.2 mm, film thickness 0.33 μm). GC-MS was with a capillary column (OV1, length 25 m, 0.32 mm i.d., film thickness 1 μm). Semipreparative HPLC was performed on μBondapak C18 column (length 10 cm, 25 mm i.d.). The elution solvent was a MeOH–H_2O mixt. (17:3) at 9 ml min^{-1} (λ 215 nm).

Extraction and isolation. Bark of *Prunus africana* was extracted with $CHCl_3$ under reflux and 2 kg of the extract were mixed with 40 kg of sand and percolated by 15 l. of cyclohexane and Et_2O successively. A portion of the ethereal fraction (8 g) was chromatographed on a Sephadex LH-20 gel column and divided into 4 frs by elution with CH_2Cl_2–MeOH (2:1). A portion of the third fraction (2.5 g) was dissolved in CH_2Cl_2 and the insoluble fraction (1.55 g) was subjected to CC on a silica gel column and eluted with gradients of CH_2Cl_2 and MeOH. The appropriate fractions (monitored by TLC and GC analysis) were combined to give four frs: A (97:3), B (19:1), C (93:7) and D (9:1). The CH_2Cl_2-soluble fr. (0.98 g) constituted the fraction E.

Fr. A (150 mg) was methylated by an excess of ethereal CH_2N_2. Evapn of the solvent *in vacuo* left a residue which was chromatographed on a silica gel column and eluted with *n*-C_6H_{14}–EtOAc (17:3) to give methyl ursolate and methyl 2α,3α-dihydroxyurs-12-en-28-oate (**1a**).

Fr. B (40 mg) was methylated with an excess of ethereal CH_2N_2 and acetylated with Ac_2O–pyridine (2:1, 6 ml). Evapn of the solvent *in vacuo* left a residue which was chromatographed on a silica gel column and eluted with *n*-C_6H_{14}–EtOAc (9:1) to yield methyl 2α,3β-diacetoxyolean-12-en-28-oate (**3a**) and methyl 3β,24-diacetoxyurs-12-en-28-oate (**4a**).

Fr. C (145 mg) was treated as for Fr. B to yield methyl 2α,3β-diacetoxyurs-12-en-28-oate (**2a**) and methyl 2α,3α,23-triacetoxyurs-12-en-28-oate (**5a**).

Fr. D (230 mg) was methylated with an excess of ethereal CH_2N_2 and acetylated with Ac_2O–pyridine (2:1, 6 ml). Evapn of the solvent *in vacuo* left a residue which was chromatographed on a silica gel column and eluted with *n*-C_6H_{14}–CH_2Cl_2–MeOH (80:20:1) to give methyl 2α,3α,24-triacetoxyurs-12-en-28-oate (**6a**).

Fr. E (980 mg) was subjected to silica gel CC and eluted with a gradient of CH_2Cl_2–MeOH to give compound **7**, a mixture of compounds **7** and **8**, and compound **9**. Compound **9** was purified by semiprep. HPLC.

Alkaline hydrolysis of **7** *and* **8**. A mixt. of **7** and **8** (10 mg) was treated by 10% NaOH aq. (5 ml). After neutralization by HCl, the reactive soln was extracted by *n*-BuOH. The aq. soln was acidifed by HCl and extracted by Et_2O. The *n*-BuOH and Et_2O extracts were evapd to dryness and subjected to GC analysis. *Cis*- and *trans*-ferulic acids and compound **4** were identified.

Alkaline hydrolysis of **9**. Compound **9** (8 mg) was treated in the same conditions of **7** and **8**. *Trans*-ferulic acid and compound **6** were identified respectively.

Compounds **1a–6a** *and* **7** *and* **8**. ^1H NMR and ^{13}C NMR (200 MHz, $CDCl_3$): data were in agreement with those described in refs. [3–5].

Compounds **6**, **6a** *and* **9**. ^1H NMR (200 MHz, pyridine-d_5): see Table 1.

Compound **10**. CI-MS (CH_4^+). *m/z* 664.9 (6) [M^+], 648 (13), 620 (3), 603 (2), 485 (9), 483 (7), 471 (9), 469 (10), 455 (18), 441 (18), 425 (14), 423 (11), 407 (16), 395 (16), 248 (11), 203 (9), 178 (23), 151 (100), 150 (49), 137 (26), 135 (12), 133 (14), 125 (41), 121 (31), 119 (18), 111 (17), 109 (13), 107 (12), 95 (14), 93 (11), 85 (21), 83 (20). EI-MS (70 eV). *m/z* 248 (17), 203 (19), 150 (72), 137 (13), 135 (79), 133 (29), 124 (13), 121 (14), 119 (21), 109 (23), 107 (46), 95 (14), 93 (14), 91 (21), 89 (9), 84 (9), 81 (23), 79 (30), 77 (41), 69 (20), 67 (25), 63 (15), 57 (22), 55 (37), 53 (28), 51 (27), 44 (100).

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