Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 453–456
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Phenolic constituents from *Drypetes armoracia*☆

Jean Wandji[a,*], François Tillequin[b], Dulcie A. Mulholland[c], Agathe D. Temgoua[a], Jean-D. Wansi[a], Elisabeth Seguin[d], Z. Tanee Fomum[a]

[a]*Department of Organic Chemistry, University of Yaoundé-1, Faculty of Science, P.O. Box 812 Yaoundé, Cameroon*
[b]*Laboratoire de Pharmacognosie, UMR/CNRS No-8638, Université René- Descartes, Faculté des Sciences Biologiques et Pharmaceutiques, 4-Avenue de l'Observatoire, Paris, France*
[c]*Department of Chemistry, Natural Products Research Group, University of Natal, Durban, South Africa*
[d]*Laboratoire de Pharmacognosie, UFR de Médecine Pharmacie Rouen, 22-Bld Gambetta, 76183 Rouen Cedex 1, France*

Received 31 July 2002; received in revised form 1 November 2002

Abstract

The methanol extract of the dried stem bark of *Drypetes armoracia* Pax & Hoffm. afforded two compounds named drypearmoracein A, (E)-4,5,6,7-tetrahydroxy-2-benzylhept-2-enoic acid and drypearmoracein B, 2,3-dihydroxy-9,10-tetrahydroanthra-1,4-quinone along with five known compounds: friedelan-3 β-ol, friedelin, friedelane-3,7-dione, drypemolundein B and β-stigmasterol. Their structures were established on the basis of spectroscopic analysis and chemical evidence.

Keywords: *Drypetes armoracia*; Euphorbiaceae; Stem bark; Drypearmoraceins A and B; Hydroxyanthraquinone

1. Introduction

Drypetes armoracia Pax & Hoffm (Euphorbiaceae) is one of more than 20 species of *Drypetes* found in Cameroon. Some of the *Drypetes* species have traditionally been used in West and Central Africa to treat various diseases including sinusitis, boils, swellings gonorrhoea and dysentery (Dalziel, 1937; Irvine, 1961; Bouquet and Debray, 1974; Walker et al., 1961). Recently, we reported the isolation of a new sesquiterpene lactone and a new friedelane-type triterpene from the stems of *D. molunduana* (Wandji et al., 2000). The total extract and the sesquiterpene lactone were found to exhibit strong anti-inflammatory and analgesic actions (Chungag-Anye et al., 2001, 2002). This background prompted us to explore the chemical constituents of the Cameroonian species, *D. armoracia*. We describe herein the isolation and the structural elucidation, on the basis of extensive NMR studies, of two new compounds: drypearmoracein A, (E)-4,5,6,7-tetrahydroxy-2-benzylhept-2-enoic acid **1** and drypearmoracein B, 2,3-dihydroxy-9,10-tetrahydroanthra-1,4-quinone **2** .

2. Results and discussion

The methanol extract of the air dried stem bark of *D. armoracia* was repeatedly subjected to column chromatography on silica gel to separate compounds **1**–**7**.

Compound **1** was obtained as colourless crystals. The molecular formula of $C_{14}H_{18}O_6$, containing 6 insaturation equivalents, was deduced by DEPT, ^{13}C NMR, CIMS and FABMS data. Its IR spectrum indicated absorption bands due to the carboxylic acid (1750 cm $^{-1}$), hydroxyl (3450 cm $^{-1}$) and double bonds C=C (1600 cm^{-1}) groups. The UV spectrum showed absorption band at λ_{max} 265.2 nm. The ^1H NMR spectrum of **1** indicated a multiplet at δ 7.27–7.34 assigned to the five protons of one monosubstituted benzene ring. It also showed three hydroxymethine protons at δ 4.82 (H-4), 3.51 (H-5) and 3.65 (H-6) and one pair of hydroxymethylene proton signals at δ 3.58 (H-7a) and 3.74 (H-7b). The offset signal at δ 11.82 (*br s*) was attributed to the COOH proton. Thus, these informations suggested that compound **1** contained four hydroxyl groups and one carboxylic acid function. The ^1H NMR spectrum of the acetylated derivative **1a** showed four singlets at δ 2.10, 2.05, 2.02 and 2.00 (3H each), assigned to the acetyl protons, confirming therefore the presence of four hydroxyl groups in **1**. In addition, the ^1H NMR

☆ Part 4 in the series Drypetes studies.

* Corresponding author. Tel.: +237-231-09-57; fax: +237-223-53-86.
E-mail address: jeanwandji@yahoo.fr (J. Wandji).

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(02)00630-1

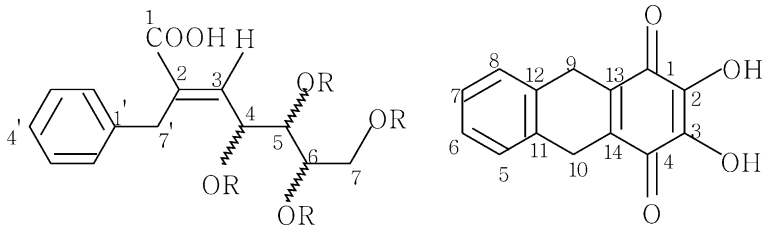
Table 1
^{13}C NMR and DEPT data[a] and HMBC connectivities of drypearmoracein A **1** (100 MHz, DMSO-d_6)

Attribution	^{13}C	DEPT	HMBC
1	160.1	C	
2	131.2	C	
3	115.1	CH	C-1, C-2, C-4, C-5, C-7′
4	64.8	CH	C-2, C-3, C-5, C-6
5	73.5	CH	C-3, C-4, C-6, C-7
6	71.4	CH	C-4, C-5, C-7
7	63.6	CH_2	C-5, C-6
1′	136.7	C	
2′	127.8	CH	C-1′, C-3′, C-4′
3′	128.5	CH	C-1′, C-2′, C-4′
4′	127.6	CH	C-2′, C-3′, C-5′ C-6′
5′	128.5	CH	C-1′, C-6′, C-4′
6′	127.8	CH	C-1′, C-5′, C-4′
7′	49.8	CH_2	C-1, C-2, C-3, C-1′, C-2′, C-6′

[a] Assignments were accomplished using ^1H COSY, HETCOR and HSQC experiments.

spectrum of **1** showed one doublet at δ 5.18 ($J = 1.20$ Hz), assigned to the CH_2 protons (H-7′) of the benzyl group linked to the sp^2 carbon (C-2) and one broad singlet at δ 6.78 assigned to one olefinic proton (H-3). The decoupled ^{13}C NMR and DEPT spectra of **1** (Table 1) showed the presence of 14 carbons. The COOH group carbon at δ 160.1 justified its linkage to the sp^2 carbon of the double bond. In addition, the ^{13}C NMR spectrum showed two double bond carbons at δ 131.1 (C-2) and 115.1 (C-3), six monosubstituted benzene ring carbons at δ 136.7 (C-1′), 128.5 (C-2′/ C-6′), 127.8 (C-4′) and 127.7 (C-3′/C-5′); the CH_2 group (C-7′) was observed at δ 49.8. The presence of four hydroxyl bearing carbons was confirmed by the chemical shifts observed at δ 64.8 (C-4), 73.5 (C-5), 71.4 (C-6) and 63.6 (C-7). Comprehensive analysis of the COSY spectrum revealed many correlations that established the successive couplings between the following protons: H-4 (δ 4.82) and H-5 (δ 3.51), H-5 and H-6 (δ 3.65), H-6 and H-7a/ H-7b (δ 3.58/ 3.74). The cross peak observed between the COOH proton (δ 11.82) and the olefinic proton (δ 6.78) suggested the *trans*-configuration of the double bond $C_2{=}C_3$ (see Fig. 1). Furthermore, the HMBC spectrum (Table 1) showed cross peaks between the COOH carbon (δ 160.1) and H-7′ (δ 5.18), H-3 (δ 6.78), confirming that the COOH group was at the *viccinal*-position with the olefinic proton. The stereochemistry of the asymmetric carbons C-4, C-5 and C-6 was not determined. From the complete spectroscopic data and the mass fragments (see experimental), compound **1** was established as (E)-4,5,6,7-tetrahydroxy-2-benzylhept-2-enoic acid, a new constituent named drypearmoracein A.

The elemental composition of compound **2**, $C_{14}H_{10}O_4$ was deduced by the CI/NH_3 MS (m/z 243 $[M+H]^+$, 260 $[M+NH_4]^+$) and EIMS (m/z 242 $[M]^+$), corresponding to 10 insaturation equivalents. The presence of a hydroxyquinonic moiety in **2** was suggested by its UV spectrum data at λ_{max} 267.2, 263.0 and 258.0 nm. This suggestion was further confirmed by the IR spectrum indicating absorption bands due to OH (3400 cm $^{-1}$), quinonic C=O (1660 cm $^{-1}$) and double bond C=C (1600 cm $^{-1}$). The ^{13}C NMR spectrum of **2** (Table 2) showed only seven carbon signals. The hydroxyquinonic moiety in **2** was confirmed by the presence of two ketonic functions at δ 184.3 (C-1 and C-4) with a very low intensity and two oxygenated quaternary carbons at δ 158.2 (C-2 and C-3). These data were comparable to those of a methoxyquinonic moiety reported for some quinone derivatives (Guntern et al., 2001). Comprehensive analysis of the seven ^{13}C NMR signals suggested the presence of one perfect symmetry in **2**. Therefore, its molecular formula $C_{14}H_{10}O_4$ contained two identical parts represented as $(C_7H_5O_2)_2$. Examination of its DEPT spectrum revealed the presence of three signals only, assigned to the protonated carbons at δ 127.4 (CH), 127.6 (CH) and 44.5 (CH_2). In addition, both the HETCOR and HSQC spectra showed three cross peaks only between the protonated carbons and the corresponding protons at δ_C 44.5/δ_H 4.42, δ_C 127.4/δ_H 7.21 and δ_C 127.6/δ_H 7.29. The ^1H NMR spectrum of **2** (Table 2) showed five signals of which four confirmed the presence of the CH_2 group protons as two singlets at δ 4.26 and 4.28 (H-9/H-10) and the CH protons at δ 7.21 (*d*, $J = 7.0$ Hz, H-5 /H-8) and 7.29 (*d*, $J = 7.0$ Hz, H-6/H-7). The fifth signal at δ 5.02 (*br. s*) was deduced to be the



1 R = H

1a R = Ac

2

Fig. 1. Structures of compounds **1** and **2**.

Table 2
^{13}C, DEPT and ^{1}H NMR data[a] for drypearmoracein B **2** in $CDCl_3$

Position	^{13}C	DEPT	^{1}H (*J*, Hz)
1/4	184.3[b]	C	
2/3	158.2	C	5.02, *br s*, OH
5/8	127.4[c]	CH	7.21, *d* (7.0)
6/7	127.6[c]	CH	7.29, *d* (7.0)
9/10	44.5	CH_2	4.26; 4.28, *s*, each
11/12	127.2	C	
13/14	139.1	C	

[a] Assignments were accomplished using ^{1}H COSY, HETCOR and HSQC.
[b] Very low intensity.
[c] Values can be reversed.

two hydroxyl groups located at the C-2 and C-3 positions in agreement with the presence of the symmetry in **2** (see Fig. 1). At these positions, the two OH groups influence by electronic effects the quinonic ketones at C-1 and C-4, and could then justify the low intensity of their signal at δ 184.3. The complete assignments were accomplished using ^{1}H–^{1}H COSY experiment. In accordance to the above spectral data, the arrangement of the elemental formula, in respect to the presence of one perfect symmetry, established the structure of compound **2** as 2,3-dihydroxy-9,10-tetrahydroanthra-1,4-quinone. The structure **2** was supported by its EIMS with some selected important fragments at *m/z* 240 [M-2H] (42), 106 [C_6H_{10}] (100) and 91 [C_7H_7] (80). Compound **2** is new anthra-1,4-quinone derivative named drypearmoracein B.

Compounds **3**–**7** were known constituents isolated for the first time from *D. armoracia*. Their ^{13}C NMR spectral data compared to those of the literature identified them as friedelan-3 β-ol **3**, friedelin **4**, friedelane-3,7-dione **5** (Mahato and Kundu, 1994), drypemolundein B **6** (Wandji et al., 2000) and β-stigmasterol **7** (Tandon et al., 1990).

3. Experimental

3.1. General

MPs were determined using a Kofler microhot stage apparatus. IR spectroscopy was performed on a Perkin-Elmer 257 spectrometer. $[\alpha]_D$ were read on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Beckman DU-600 spectrometer. MS were registered on a Micromass Q-Tof instrument. NMR experiments were performed on a Varian Gemini 400 MHz instrument and a Bruker AC 300 spectrometer. The solvents used for NMR were $CDCl_3$ and DMSO-d_6, also considered in each case as internal references. Si gel 60H (5–40 μm), 60C (20–40 μm) and 60 (240–400 mesh) were used for CC under compressed air (300 mbar) while precoated Si gel 60 F_{254} aluminium plates were used for TLC.

3.2. Plant material

The stem bark of *Drypetes armoracia* Pax and Hoffm was collected from the Dja forest, (East Province of Cameroon) in February 1998. The herbarium specimen documenting the collection has been deposited in the National Herbarium, Yaoundé, Cameroon (Ref. RL.12120).

3.3. Extraction and isolation

Air-dried and powdered stem bark of *Drypetes armoracia* (3.4 kg) was extracted with MeOH (3×4 l) for (3×48 H) at room temperature. After the evaporation of the solvent under reduced pressure, the crude MeOH extract (190.0 g) was obtained. Part of the extract (150.0 g) was chromatographed on Si gel 60 (230–400 mesh) (600 g) using hexane, hexane–EtOAc, EtOAc–MeOH and MeOH in order of increasing polarity . A total of 80 fractions (400 ml each) were eluted. On the basis of the TLC, the fractions were combined into five main series of fractions [A–E]: A (8.6 g) (fractions 5–18) and B (1.3 g) (fractions 19–40), both series eluted with EtOAc–hexane from 25:75 to 30:70; C (5.6 g) (fractions 41–63), eluted with EtOAc–hexane from 40:60 to 100:0; D (12.2 g) (fractions 64–74), eluted with MeOH–EtOAc from 5:95 to 50:50 and E (40.0 g) (fractions 75–80), eluted with MeOH–EtOAc from 60:40 to 100:0. Repeated CC of series A (8.6 g) on Si gel 60C (20–40 μm) using hexane–EtOAc in increasing percentages yielded compounds **3** (350 mg), **4** (200 mg), **5** (300 mg), **6** (40 mg) and **7** (120 mg). Further CC of series B (1.3 g) on Sigel 60 H (5–40 μm) using hexane–EtOAc in increasing proportions yielded compound **2** (30 mg). Further CC of series D (12.2 g) on Si gel 60H (5–40 μm) using EtOAc–MeOH in increasing polarities afforded compound **1** (220 mg).

3.3.1. Drypearmoracein A [(*E*)-4,5,6,7-tetrahydroxy-2-benzylhept-2-enoic acid] **1**

Colourless crystals; mp 250 °C; $[\alpha]_D$ +27° (MeOH, *c* 0.750); IR (KBr) λ_{max} (cm^{-1}): 3450 (OH), 1750 (COOH), 1600 (C=C); UV [MeOH] nm (log ε): 265.2 (0.7904); CI/NH_3 MS *m/z* 283 $[M+H]^+$, 300 $[M+NH_4]^+$; FAB MS *m/z* (rel. int.): 289 $[M+Li]^+$(71.2), 283 $[M+H]^+$, 280 (27.3), 253 (28.1), 252 (100.0), 246 (47.6), 243 (32.0), 226 (40.6); EIMS, *m/z* 274, 262, 241, 203, 201, 115, 91; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.82 (1H, *br s*, COOH), 7.27–7.34 (5H, m, C_6H_5–), 6.78 (1H, *br s*, H-3), 5.18 (2H, *d*, *J* = 1.20 Hz, H-7′), 4.82 (1H, *br s*, H-4), 3.74 (1H, *dd*, *J* = 2.92, 10.98 Hz, H-7a), 3.65 (1H, *dd*, *J* = 2.65, 8.15 Hz, H-6), 3.58 (1H, *dd*, *J* = 2.92, 10.98 Hz, H-7b), 3.51 (1H, *d*, *J* = 8.15 Hz, H-5); ^{13}C

NMR (100 MHz, DMSO-d_6) and HMBC connectivities: see Table 1.

Acetylation of **1**: 10.0 mg of **1** was dissolved in the Ac_2O–pyridine mixture (1:1 ml) and heated at 60 °C for 24 h. After working, the crude product was purified on Si gel 60H to yield one acetylated derivative **1a** (12.5 mg, 78%).

3.3.2. Acetyldrypearmoracein A [(E)-4,5,6,7-tetraacetoxy-2-benzylhept-2-enoic acid] ***1a***

Amorphous solid; CI/NH_3 MS, *m/z* 451 $[M+H]^+$; M.F. $C_{22}H_{26}O_{10}$; ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.80 (*br s*, COOH), 7.30–7.35 (5H, *m*, Ar-), 6.85 (*br s*, H-3), 5.20 (*d*, $J=1.20$ Hz, H-7′), 5.12 (*br s*, H-4), 4.04 (*dd*, $J=2.92$, 10.00 Hz, H-7a), 4.02 (*dd*, $J=2.65$, 8.15 Hz, H-6), 4.00 (*dd*, $J=2.92$, 10.00 Hz, H-7b), 3.98 (*d*, $J=8.15$ Hz, H-5), 2.10 (3H, *s* Ac)), 2.05 (3H, *s*, Ac), 2.02 (3H, *s*, Ac), 2.00 (3H, *s*, Ac) .

3.3.3. Drypearmoracein B [2,3-dihydrxy-9,10-tetrahydro-anthra-1,4-quinone] ***2***

White crystals; mp 160 °C; IR (KBr) λ_{max} (cm^{-1}) : 3400 (OH), 1660 (C=O), 1600 (C=C); UV ($CHCl_3$) nm (log ε): 267.2 (0.1015), 263.0 (0.1307), 258.0 (0.1667), 252.6 (0.1450), 236.0 (0.0782); EIMS *m/z* (rel. int.): 242 $[M]^+$ (5), 241 $[M-H]^+$(7), 240 $[M-2H]^+$(42), 149 (23), 107 (12), 106 (100), 104 (14), 91 (80), 79 (32), 65 (31), 51 (20); ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) values: see Table 2.

Compounds **3**, **4** and **5** were identical to friedela-3β-ol, friedelin and friedelane-3,7-dione respectively by comparison of their ^{13}C NMR data with those reported (Mahato and Kundu, 1994).

Compound **6** was identical to drypemolundein B (friedelane-3-11-dione) previously isolated from *D. molunduana* (Wandji et al., 2000).

Compound **7** was identified to β-stigmasterol by the comparison of its NMR spectral data to the literature values (Tandon et al., 1990).

The friedelane-type triterpenes and sterols represented more than 50% of the constituents isolated from *D. armoracia*.

Acknowledgements

One of the authors (J. Wandji) is grateful for IFS grant No F/2624-2 (Sweden), for the sponsorship of the ''Université René- Descartes Paris V, France'' during his research visit in Paris, and for the financial support from the OPCW (The Netherlands), through the Internship at the University of Natal, Durban, South Africa. Thanks are due Mr. Dilip Jagjivan for the measurements of some NMR spectra and to Mr. Zacharie D. Nzooh for the identification and the collection of this plant.

References

Bouquet, A., Debray, L., 1974. Plantes Médicinales de la Côte-d'Ivoire; Travaux et Documents de l'ORSTOM No. 32, pp. 82–87.

Chungag Anye, N.B., Njamen, D., Dongmo, A.B., Wandji, J., Nguelefack, T.B., Wansi, J.D., et al., 2001. Anti-inflammatory and analgesic properties of the stem extract of *Drypetes molunduana* Pax and Hoffm. (Euphorbiaceae) in rats. Pharmaceutical and Pharmacological Letters 11, 61–63.

Chungag-Anye, N.B., Njamen, D., Wandji, J., Fomum, T.Z., Dongmo, A., Nguelefack, T.B., et al., 2002. Anti-inflammatory and analgesic effects of Drypemolundein A, a sesquiterpene lactone from *Drypetes molunduana*. Pharmaceutical Biology 40 (in press).

Dalziel, J.M., 1937. The Useful Plants of West Tropical Africa. The Crown Agents for the Colonies, London.

Guntern, A., Ioset, J.-R., Queiroz, E.F., Foggin, C.M., Hostettmann, K., 2001. Quinones from *Heliotropium ovalifolium*. Phytochemistry 58, 631–635.

Irvine, R.F., 1961. Woody Plant of Ghana. Oxford University Press, London.

Mahato, S.B., Kundu, A.P., 1994. ^{13}C NMR spectra of pentacyclic triterpenoids. A compilation and some salient features. Phytochemistry 37, 1517–1575.

Tandon, M., Shukla, Y.N., Thakur, R.S., 1990. Steroid glycosides from *Asparagus adscendens*. Phytochemistry 29, 2957–2959.

Walker, A.R., Sillans, R., Trochain, J.L., 1961. Les Plantes Utiles du Gabon. Ed. Paul Lechevalier 12-Rue de Tournon Paris VI, pp. 165–166.

Wandji, J., Wansi, J.D., Fuendjiep, V., Dagne, E., Mulholland, D.A., Tillequin, F., et al., 2000. Sesquiterpene lactone and friedelane derivative from *Drypetes molunduana*. Phytochemistry 54, 811–815.