PERGAMON

Phytochemistry 54 (2000) 811–815

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Sesquiterpene lactone and friedelane derivative from *Drypetes molunduana* ☆

J. Wandji[a,*], J.D. Wansi[a], V. Fuendjiep[b], E. Dagne[c], D.A. Mulholland[d], F. Tillequin[e], Z.T. Fomum[a], B.L. Sondengam[a], B.C. Nkeh[f], D. Njamen[f]

[a]*Department of Organic Chemistry, University of Yaounde I, Faculty of Science, P.O. Box 812, Yaounde, Cameroon*
[b]*Centre de Recherche en Plantes Médicinales et Médecine Traditionnelle, IMPM, BP 193, Yaoundé, Cameroon*
[c]*Department of Chemistry, Addis Ababa University, Faculty of Science, P.O. Box 30270, Addis Ababa, Ethiopia*
[d]*Department of Chemistry, University of Natal, Durban 4041, South Africa*
[e]*Laboratoire de Pharmacognosie, URA au CNRS No 1310, Université René Descartes, Faculté de Pharmacie, 4-Avenue de l'Observatoire, F-75006 Paris, France*
[f]*Department of Biology and Animal Physiology, University of Yaounde I, Faculty of Science, P.O. Box 812, Yaounde, Cameroon*

Received 26 September 1999; received in revised form 7 January 2000

Abstract

A new sesquiterpene lactone, drypemolundein A and a new friedelane derivative, drypemolundein B, along with seven known compounds have been isolated from the whole stems of *Drypetes molunduana* Pax and Hoffm. Their structures were established on the basis of one- and two-dimensional NMR, homo- and hetero-nuclear spectroscopic evidence.

Keywords: *Drypetes molunduana*; Euphorbiaceae; Stems; Drypemolundeins A and B; Sesquiterpene lactone; Friedelane; Triterpenoids; Lignan

1. Introduction

The genus *Drypetes* (Euphorbiaceae) comprises many species in Africa with more than 20 being identified in Cameroon. These plants are well known in folk medicine of West and Central Africa; many are used to treat various diseases including gonorrhoea, toothache, dysentery, coryza, sinusitis, boils and swellings (Dalziel, 1937; Irvine, 1961; Bouquet and Debray, 1974; Walker et al., 1961). Up to the present, chemical studies have been reported on two species: *D. roxburghii* (Sipahimalani et al., 1994) and *D. gossweileri* (Dupont et al., 1997). Recently, our investigations on the aqueous extract of the Cameroonian medicinal species, *D. molunduana* Pax and Hoffm, revealed its potent anti-inflammatory and analgesic actions (Nkeh et al., 1999).

As a continuation of our studies on the same species, we now report in this paper the isolation and structural elucidation of a new furanosesquiterpene lactone drypemolundein A **1** and a new friedelane derivative, drypemolundein B **2** along with seven known constituents, comprising six pentacyclic triterpenoids (**3**–**8**) and one lignan **9**.

2. Results and discussion

Ground, air dried stems of *D. molunduana* were extracted with CH_2Cl_2–MeOH (1/1). Repeated column chromatography of the total extract yielded compounds **1**–**9**. Analysis by ^{1}H-, ^{13}C- and 2D-NMR spectroscopy led to the determination of their structures.

Compound **3** was found to be friedelane-3,7-dione

☆ Part 2 in the series "*Drypetes* studies".

* Corresponding author. Fax: +237-317-588.
E-mail address: cmfi@camnet.cm (J. Wandji).

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00040-6

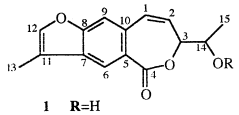
which has been isolated previously from *Putranjiva roxburghii* (Sengupta et al., 1968). Compounds **4**–**8** were identified as olean-12-ene derivatives, namely erythrodiol, 3β-acetoxyolean-12-en-28-oic acid, hederagenin and bayogenin acid, respectively, all in agreement with the results reported (Mahato and Kundu, 1994). Compound **9** was found to be (−)-syringaresinol reported by Vermes et al. (1991).

Compound **1**, drypemolundein A, was obtained as white crystals. Its molecular formula $C_{15}H_{14}O_4$ was deduced from the CI and the EI mass spectra which indicated the respective ions at m/z 259 $[M + H]^+$, 276 $[M + NH_4]^+$ and m/z 258 $[M]^+$. IR spectrum of compound **1** showed absorption bands at 3450 (free OH), 1750 (COOR) and 1600 cm^{-1} (C=C) suggesting that its skeleton contained a free hydroxyl group and an ester function. Its UV (MeOH) spectrum indicated an intense absorption at 263.4 nm suggesting the presence of a conjugated chromophore. The ^{1}H-NMR spectrum showed characteristic signals of one methylfurano entity at δ 7.49, a quartet ($J = 1.3$ Hz) assigned to the proton H-12 and at δ 2.26, a doublet ($J = 1.3$ Hz) ascribed to the methyl group (CH_3-13). This hypothesis was confirmed by the ^{13}C-NMR spectrum of **1** which showed a signal at δ 7.8 assigned to C-13 and another at δ 144.0 attributed to the oxygenated sp^2 C-12 in agreement with the results reported (Abdo et al., 1992). Furthermore, the ^{1}H-NMR showed signals corresponding to two oxymethine protons, at δ 4.28, a multiplet assigned to H-3 and δ 4.12, a doublet of quartet ($J = 6.3$ and 6.8 Hz) ascribed to H-14. It also indicated a doublet of doublet ($J = 10.2$ and 1.5 Hz) at δ 6.98 and a multiplet at δ 6.20 assigned to two olefinic protons H-1 and H-2, respectively. The presence of the oxymethine carbons was also confirmed in the ^{13}C-NMR spectrum which showed signals at δ 78.3 and δ 67.9 assigned to C-3 and C-14, respectively. The signal at δ 170.0, characteristic of the carbonyl carbon in the lactone group, was assigned to C-4. Furthermore, examination of DEPT spectrum revealed the presence of two methyl groups and seven methines corresponding to a total of 13 protons which were completely assigned on the basis of the 2D HETCOR and COSY spectra. The last proton corresponded to the single hydroxyl group in **1**. The COSY spectrum indicated a correlation between H-13 (δ 2.26) and H-12 (δ 7.49), confirming the presence of the methylfurano entity in **1**. It also showed many correlations which established the successive couplings between the following protons: H-1 (δ 6.98) and H-2 (δ 6.20), H-2 and H-3 (δ 4.28), H-3 and H-14 (δ 4.12), H-14 and H-15 (δ 1.26). On the other hand, the two singlets observed on the ^{1}H-NMR spectrum at δ 8.24 and δ 7.92 did not possess any correlations in the COSY spectrum. This information suggested that the two protons were located in the *para* positions C-5 and C-9, respectively, on the benzene ring.

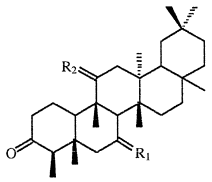
All the above spectroscopic data were in agreement with four possible structures containing one benzene ring on which one methylfurano entity and one lactone ring with seven or eight links are fused. In order to establish the exact structure, the 2D NOE (NOESY) technique was used, and in this spectrum, in addition to many correlations already indicated in the COSY spectrum, two more significant correlations were observed between δ 8.24 (H-6) and δ 2.26 (CH_3-13), and between δ 7.32 (H-9) and δ 6.98 (H-1). These results confirmed that the methyl group at C-13 is spatially close to H-6 (δ 8.24). In addition, analysis of the HMBC spectrum showed that the carbonyl at C-4 (δ 170.0) correlated with the protons H-6 (δ 8.24) and H-3 (δ 4.28). There were also correlations between the proton H-9 (δ 7.32) and the carbons C-1 (δ 136.0), C-5 (δ 125.9) and C-7 (δ 129.6). Furthermore, the downfield shift of the proton H-6 (δ 8.24) could be due to the *ortho* position of the carbonyl in the lactone function. The presence of asymmetric carbons (C-3 and C-14) in **1** justified its optical rotation, although its stereochemistry was not determined. From the above spectroscopic studies and the mass fragmentation (Scheme 1), **1** was established as a furanosesquiterpene lactone. It is a new skeleton in which the methyl-15 has probably transferred from C-3 to C-14.

Compound **2**, drypemolundein B, was obtained as a white powder. Its molecular formula $C_{30}H_{48}O_2$ was deduced from the CI and the EI mass spectra which showed the respective ions at m/z 441 $[M + H]^+$, 458 $[M + NH_4]^+$ and m/z 440 $[M]^+$. Its ^{1}H-NMR, ^{1}H–^{13}C COSY and DEPT spectra suggested a triterpene skeleton containing two carbonyl carbons (δ 212.1 and 214.2). From the EI mass spectrum, the molecular ion at m/z 440 and the characteristic peaks at m/z 288 and 205 units suggested a friedelin type triterpene, with the two carbonyl groups in the A, B and C rings and the absence of oxygen functions in the D and E rings. Furthermore, the peak at m/z 301 resulted from the cleavage involving hydrogen transfer from C-12 as reported (Sengupta et al., 1968). From the biosynthetic consideration, the first ketonic function in **2** is located at the C-3 position (Boiteau et al., 1964). Further examination of the mass spectral fragmentation pattern revealed a peak at m/z 248 $[C_{17}H_{28}O]^+$ suggesting the second carbonyl function on the C ring, notably at the C-11 position. This hypothesis was confirmed in the ^{1}H-NMR spectra of both compounds **2** and **3**: the singlet observed at δ 2.55 corresponding to H-8 in compound **3** (Sengupta et al., 1968) was absent in compound **2**. On the other hand, the ^{1}H-NMR spectrum of **2** indicated an AB system signals at δ 2.62 (*d*, $J = 11.4$ Hz) and 2.01 (*d*, $J = 11.4$ Hz) assigned to the two diastereotopic protons at the C-12 position. Fur-

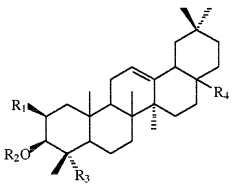
thermore, examination of the 2D NOE (NOESY) spectrum for the stereochemistry of structure **2** showed that a methyl group at δ 1.39 (H-27) correlated with a methine at δ 2.38 (H-8). The respective methyl groups at δ 0.90 (H-25) and δ 0.88 (H-26) correlated with δ 2.62 (H-12). These observations revealed that H-8 and CH_3-27 have α-configuration. From the above spectroscopic studies, **2** was established as friedelane-3,11-dione.



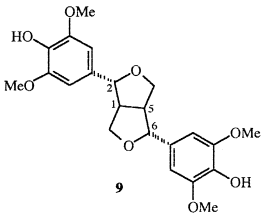
1 R=H
1a R=Ac



	R_1	R_2
2	H_2	O
3	O	H_2



	R_1	R_2	R_3	R_4
4	H	H	CH_3	CH_2OH
5	H	Ac	CH_3	COOH
6	H	H	CH_3	COOH
7	H	H	CH_2OH	COOH
8	OH	H	CH_2OH	COOH



9

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 25 spectrometer. MS were registered in Nermag R10-10C spectrometer. NMR experiments were performed on a Varian Gemini 300 MHz instrument and on a Bruker AC300 spectrometer. ^{1}H-NMR spectra were determined at 300 MHz and ^{13}C-NMR spectra at 75 MHz. Si gel 60H (5–40 μm), 60C (20–40 μm) and 60 (230–400 mesh) were used for CC under compressed air (300 mbar), while precoated Si gel 60F 254 plates were used for TLC and prep. TLC. The solvents used for spectral determination were $CDCl_3$-TMS (NMR) and $CHCl_3$ ($[\alpha]_D$).

3.2. Plant material

The whole stems of *D. molunduana* Pax and Hoffm were collected at Mintima locality, South Cameroon, in August 1997. The herbarium specimen documenting the collection has been deposited in the National Herbarium, Yaounde (Ref. No. 4926 HNC).

3.3. Extraction and isolation

Powdered, air-dried stems of *D. molunduana* Pax and Hoffm (1.0 kg) were extracted with CH_2Cl_2–MeOH (1/1) at room temperature. After removal of the solvents by evaporation under reduced pressure, the crude extract (80.0 g) was chromatographed on Si gel 60 (230–400 mesh) using hexane, CH_2Cl_2 and MeOH in increasing polarity. TLC led to the combination of the resulted fractions in four series A–D. Further CC of series B (6.0 g) using hexane, $CHCl_3$ and AcOEt in increasing proportions yielded compounds **1** (80 mg), **2** (25 mg), **3** (10 mg), **4** (26 mg), **5** (20 mg), **6** (70 mg) and **9** (30 mg). Further CC of series C (90 g) using $CHCl_3$–MeOH in increasing polarity yielded compounds **7** (50 mg) and **8** (20 mg).

3.3.1. Drypemolundein A *1*

White crystals; mp 148–150°C; $[\alpha]_D^{25}$ −137.0° ($CHCl_3$, *c* 1.01); IR (KBr) ν_{max} (cm^{-1}): 3450 (OH), 1750 (COOR), 1600 (C=C); UV [MeOH] nm (log ε): 263.4 (3.88); CI/NH_3 MS, *m/z* 259 $[M + H]^+$, 276 $[M + NH_4]^+$; EIMS (probe) 70 eV, *m/z* (rel. int.): 258 $[M]^+$ (3.2), 215 (9.4), 214 (78.0), 197 (2.5), 185 (100.0), 158 (11.9), 157 (18.8), 130 (4.4), 128 (29.4); ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$): see Table 1; NOESY interactions: see Fig. 1.

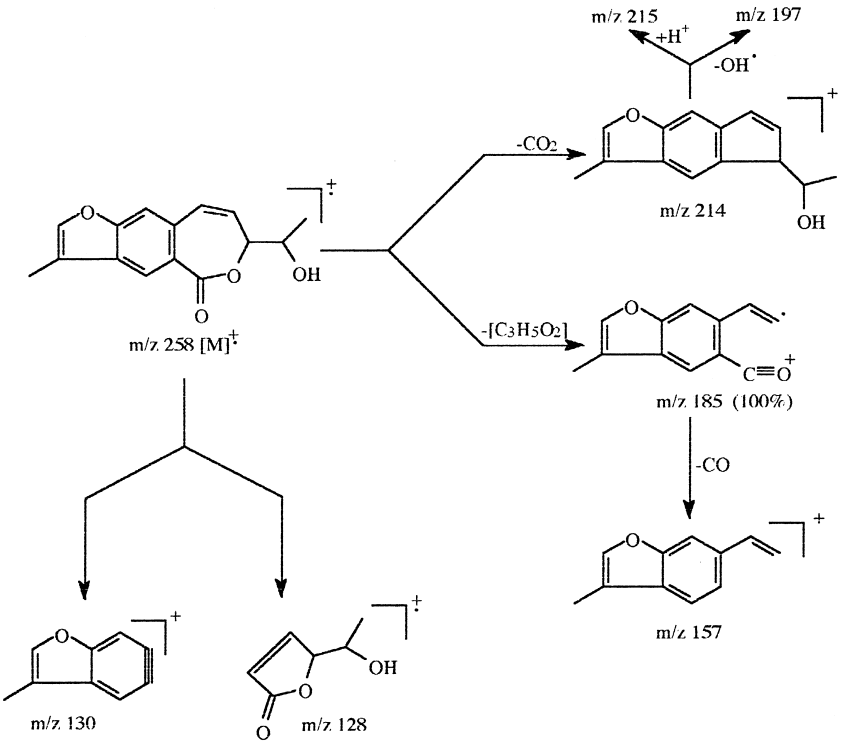
Acetylation of **1**: 10.0 mg of **1** was dissolved in the Ac_2O–pyridine mixture (1:1 ml) and heated (60°C) for 24 h. After working up, the crude product was chromatographed on Si gel 60H to yield one acetylated derivative **1a** (8.0 mg, 68.8%).

3.3.2. Acetyldrypemolundein A *1a*

White powder; CI/NH_3 MS, *m/z* 301 $[M + H]^+$; M.F. $C_{17}H_{16}O_5$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.25 (1H, *s*, H-6), 7.50 (1H, *q*, $J = 1.2$ Hz, H-12), 7.35 (1H, *s*, H-9), 7.00 (1H, *dd*, $J = 10.3$; 1.5 Hz, H-1), 6.25 (1H, *m*, H-2), 4.40 (1H, *m*, H-3), 4.30 (1H, *s*, H-9), 2.43 (3H, *s*, Ac), 2.27 (3H, *d*, $J = 1.2$ Hz, CH_3-13) and 1.32 (3H, *d*, $J = 6.2$ Hz, CH_3-15).

3.3.3. Drypemolundein B *2*

White powder; mp 290–292°C; $[\alpha]_D^{25}$ −8.0° ($CHCl_3$, *c* 1.0); IR (KBr) ν_{max} (cm^{-1}): 1705 (C=O), 1710 (C=O), 1390, 1385, 1370 (gem-dimethyl); CI/NH_3 MS, *m/z* 441 $[M + H]^+$, 458 $[M + NH_4]^+$; EIMS (probe) 70 eV, *m/z* (rel. int.): 440 $[M]^+$ (19.0), 301



Scheme 1. Mass spectral fragmentation of drypemolundein A **1**.

$[C_{20}H_{29}O_2]^+$ (35.7), 288 $[C_{29}H_{28}O_2]^+$ (47.6), 248 $[C_{17}H_{28}O]^+$ (30.9), 205 $[C_{15}H_{15}]^+$ (26.2); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.62 (1H, *d*, $J = 11.4$ Hz, H-12 ax), 2.01 (1H, *d*, $J = 11.4$ Hz, H-12 eq), 1.39 (3H, *s*, CH_3-29), 0.91 (3H, *s*, CH_3-28), 1.04 (3H, *s*, CH_3-29), 0.91 (3H, *s*, CH_3-30), 0.90 (3H, *s*, CH_3-25), 0.88 (3H, *s*, CH_3-26), 0.86 (3H, *d*, $J = 3.1$Hz, CH_3-23) and 0.73 (3H, *s*, CH_3-24); ^{13}C-NMR (75 MHz, $CDCl_3$): see Table 2.

Table 1
^{1}H- and ^{13}C-NMR assignments for drypemolundein A **1**

Attribution	^{13}C ($CDCl_3$)	^{1}H ($CDCl_3$) *J* (Hz)
1	13.60	6.98 (*dd*, $J = 10.3, 1.5$)
2	129.5	6.20 (*m*, $J = 10.2, 5.9, 4.3$)
3	78.5	4.28 (*m*, $J = 6.8, 5.9, 4.3$)
4	170.0	
5	125.9	
6	125.1	8.24 (*s*)
7	129.6	
8	156.9	
9	111.3	7.32 (*s*)
10	131.8	
11	116.4	
12	144.0	7.49 (*q*, $J = 1.3$)
13	7.8	2.26 (*d*, $J = 1.3$)
14	67.9	4.12 (*dq*, $J = 6.80, 6.3$)
15	18.2	1.26 (*d*, $J = 6.33$)
OH		2.35 (*br*, *s*)

3.3.4. *Friedelane-3,7-dione* **3**

White powder; mp 286°C; IR (KBr) ν_{max} (cm^{-1}): 1710 (C=O); DIC/NH_3 MS, *m/z* 441 $[M + H]^+$; ^{13}C-NMR spectral data (see Table 2) were in agreement with those reported by Mahato and Kundu (1994).

3.3.5. *Erythrodiol* **4**

White crystals; mp 236°C; IR, ^{1}H-NMR and EIMS were identified to authentic compound; ^{13}C-NMR spectral data were in agreement with those reported by Mahato and Kundu (1994).

3.3.6. *3β-Acetoxyolean-12-en-28-oic acid* **5**

White powder; mp 261–262°C; IR, ^{1}H- and ^{13}C-NMR data were in agreement with the authentic compound (Mahato and Kundu, 1994).

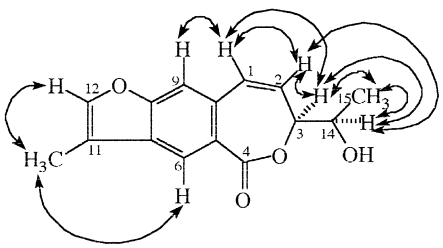


Fig. 1. NOESY correlations in compound **1**.

Table 2
^{13}C-NMR assignments for drypemolundein B **2** and friedelane-3,7-dione **3**

No.	**2** ($CDCl_3$)	**3** ($CDCl_3$)
1	22.2	21.7
2	40.8	40.8
3	212.1	211.5
4	57.9	57.9
5	42.2	47.1
6	41.0	56.1
7	18.4	211.2
8	53.0[a]	63.3
9	55.5	42.3
10	53.1[a]	59.1
11	214.2	35.6
12	51.2	29.9
13	44.0	39.4
14	43.8	37.5
15	31.6	31.6
16	36.1	36.3
17	29.6	30.1
18	36.4	41.8
19	35.4	34.9
20	28.3	28.1
21	33.0	32.9
22	38.9	38.7
23	6.8	6.8
24	14.5	15.0
25	18.1	18.2
26	19.0	19.2
27	19.8	19.6
28	31.8	32.1
29	31.7	31.7
30	34.2	34.5

[a] Values can be reversed.

3.3.7. *Oleanolic acid* **6**

White crystals; mp 304°C; EIMS, IR, ^{1}H- and ^{13}C-NMR agreed with the authentic compound (Mahato and Kundu, 1994).

3.3.8. *Hederagenin* **7**

White powder; mp 326°C; DIC/NH_3 MS, m/z 490 $[M + NH_4]^+$; ^{1}H- and ^{13}C-NMR data agreed with the authentic compound (Mahato and Kundu, 1994).

3.3.9. *Bayogenin acid* **8**

White powder; mp 337–340°C; MS, ^{1}H- and ^{13}C-NMR spectral data agreed with those reported by Mahato and Kundu (1994).

3.3.10. *(−)-Syringaresinol* **9**

Yellow crystals; mp 177°C, IR, MS, ^{1}H- and ^{13}C-NMR spectral data were in agreement with the results obtained (Barbara et al., 1991).

Acknowledgements

This research was funded by a grant from the International Foundation for Science (IFS) and the University of Yaounde I fund. Thanks are due to Mr. Dilip Jagjivan for measurements of some of the NMR spectra and to Mr. Nole Tsabang for the identification and collection of this plant.

References

Abdo, S., Bernadi, M.D., Marinoni, G., Mellerio, G., Samaniego, S., Vidari, G., et al., 1992. Furanoeremophilanes and other constituents from *Senecio canescens*. Phytochemistry 31 (11), 3937–3941.

Boiteau, P., Pasich, B., Ratisimananga, A.R., 1964. Les Triterpénoides en Physiologie Animale et Végétale. Gauthier Villars, Paris.

Bouquet, A., Debray, L., 1974. Plantes Médicinales de la Côte-d'Ivoire; Travaux et Documents de l'ORSTOM N-32., pp. 83–87.

Dalziel, J.M., 1937. *The useful Plantes of West Tropical Africa*. The Crown Agents for the Colonies, London, pp. 140–141.

Dupont, M.P., Librés, G., Delaude, C., Tchissambou, L., Gastmans, J.P., 1997. Sterolic and triterpenoidic constituents of stem bark of *Drypetes gossweileri*. Planta Med 63, 282–284.

Irvine, R.F., 1961. Woody plants of Ghana. Oxford University Press, London, pp. 223–226.

Mahato, S.B., Kundu, A.P., 1994. ^{13}C-NMR spectra of pentacyclic triterpenoids. A compilation and some salient features. Phytochemistry 37 (6), 1517–1575.

Nkeh, B.C., Wandji, J., Njamen, D., Nguelefack, T.B., Yewah, M.P., Kamanyi, A., et al., 1999. Anti-inflammatory and analgesic properties of the stem extract of *Drypetes molunduana*. *Rats. Phytother. Res.*, submitted.

Sengupta, P., Chakraborty, A.K., Duffield, A.M., Durham, I.J., Djerassi, C., 1968. Chemical investigation on *Putranjiva roxburghii*. The structure of a new triterpene, Putranjivadione. Tetrahedron 24 (3), 1205–1213.

Sipahimalani, A., Nörr, H., Wagner, H., 1994. Phenylpropanoid glycosides and tetrahydrofuranofuranlignan glycosides from the adaptogenic plant drugs *Tinospora cordifola* and *Drypetes roxburghii*. Planta Med 60, 596–597.

Vermes, B., Seligmann, O., Wagner, H., 1991. Synthesis of biologically active tetrahydrofuranofuranlignan-(synringin, pinoresinol)-mono- and bis-glucosides. Phytochemistry 30 (9), 3087–3089.

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.