PERGAMON

Phytochemistry 51 (1999) 829–832

PHYTOCHEMISTRY

An isoflavan-quinone and a flavonol from *Millettia laurentii*[1]

Pierre Kamnaing[b], Samuel N. Y. Fanso Free[c], Augustin E. Nkengfack[a], Gabriel Folefoc[a], Zacharias Tanee Fomum[a,*]

[a]*Department of Organic Chemistry, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon*
[b]*Department of Chemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon*
[c]*Department of Chemistry, University of Buea, P.O. Box 63, Buea, Cameroon*

Received 19 October 1998; received in revised form 11 January 1999

Abstract

A new isoflavan-quinone, 3′,6′-diketo-7-hydroxy-8,2′,4′-trimethoxyisoflavan, named laurentiquinone and a new flavonol, 3,7,4′-trihydroxy-3′,5′-dimethoxyflavone, named laurentinol, have been isolated from the heartwood of *Millettia laurentii* in addition to two known isoflavones, calycosin and glyricidin. The structures of the new compounds were elucidated from spectral studies. ^{13}C-NMR spectral data of the known isoflavones are reported here for the first time.

Keywords: *Millettia laurentii*; Leguminosae; Wood; Isoflavan-quinone; Flavonol; Isoflavones

1. Introduction

In the previous papers (Ngamga et al., 1993; Kamnaing, Fanso Free, Fomum, Martin, & Bodo, 1994; Ngamga, Fanso Free, Fomum, Martin, & Bodo, 1994; Mbafor, Atchade, Nkengfack, Fomum, & Olov, 1995Feundjiep, Nkengfack, Fomum, Sondengam, & Bodo, 1998a; Fuendjiep, Nkengfack, Fomum, Sondengam, & Bodo, 1998b), we reported a series of guanidine alkaloids (Ngamga et al., 1993; Kamnaing et al., 1994; Ngamga et al., 1994), isoflavonoids (Fuendjiep et al., 1998a, 1998b) and flavones (Mbafor et al., 1995) from the seeds and stem bark of *Millettia* species found in Cameroon and which have proven to possess insecticidal, piscicidal and molluscicidal activities (Teesdale, 1954; Singhal, Sharma, Baruah, Govindam, & Herz, 1982; Mbenkum, 1986). In a continuing study of these plants, we report in the present paper the isolation and structure elucidation of two new flavonoid metabolites designated laurentiquinone (**1**) and laurentinol (**2**) from the wood of *Millettia laurentii*, along with two known isoflavones, calycosin (**3**) and gliricidin (**4**).

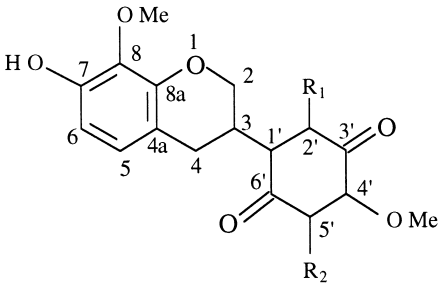
2. Results and discussion

The air-dried and powdered wood of *Millettia laurentii* was extracted successively with *n*-hexane, chloroform and methanol. The MeOH extract was subjected to column chromatography over alumina (activity 1), using gradient elution from CH_2Cl_2 to EtOAc and a mixture of EtOAc–MeOH to afford laurentiquinone (**1**) in addition to known calycosin (**3**) and gliricidin (**4**). Laurentinol (**2**), on the other hand, was obtained from column chromatography over silica gel of the $CHCl_3$ extract.

Laurentiquinone (**1**), m.p. 205–207°C, was obtained as orange prisms and reacted positively with $FeCl_3$ reagent. Its molecular formula was established as $C_{18}H_{18}O_7$ by HREIMS and ^{13}C-NMR analysis (Table 1). The compound's orange colour and comparison of its UV (λ_{max} (MeOH) (log ε) 266 (4.10), 385 (3.06) nm), IR (ν_{max}(KBr) 1680 and 1650 (conj. C=O) cm^{-1}) and ^{1}H-NMR Table 1 spectral data with those

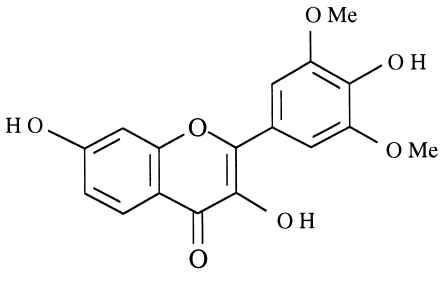
* Corresponding author.
[1] Part 10 in the series "The Millettia of Cameroon".

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00043-6

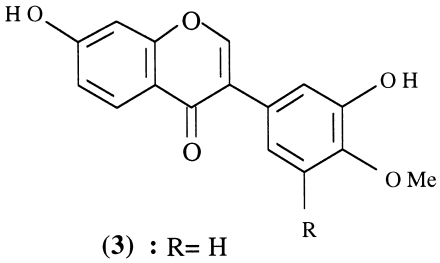


(1) : R_1= O Me, R_2= H

(5): R_1= H, R_2= O Me



(2)



(3) : R= H

(4) : R= O H

of known mucroquinone (Kurosawa, Ollis, Sutherland, Gottlieb, & Oliveira, 1978) and several more recently isolated analogues (Sibata, & Sumio, 1978) showed a close similarity, indicating **1** to be a member of an isoflavan-quinone class of compounds (Kurosawa et al., 1978; Sibata, & Sumio, 1978). In fact, the spectral data (IR, UV ^{1}H-NMR and MS) of laurentiquinone (**1**) were almost superimposable with those of amorphaquinone (**5**), an isoflavan-quinone isolated from *Amorpha fructicosa* (Sibata, & Sumio, 1978), indicating that the two compounds are isomers having the same A-ring with one methoxyl group at C-8 position, one hydroxyl group at C-7 and an AB spin system of two aromatic protons at C-5 and C-6 positions. Although their rings B also had the same substituents, quinonyl moiety, two methoxyl groups and one quinonyl proton, the positions of the two methoxyl groups on that ring were different. While in compound (**5**), the two methoxyl groups were in positions C-4′ and C-5′, in laurentiquinone (**1**), they were located at positions C-2′ and C-4′. This was deduced from long range ^{1}H–^{1}H COSY spectrum of **1** which showed no correlation peaks between the methoxyl signals, but strong cross peaks for coupling between the low quinonyl proton H-5′ at δ 5.88 and one methoxyl resonance at δ 3.80 ppm. Thus, the structure of laurentiquinone (**1**) is 3′,6′-diketo-7-hydroxy-8,2′,4′-trimethoxyisoflavan.

The second compound, laurentinol (**2**), was isolated as green prisms and reacted positively with $FeCl_3$ reagent. It was formulated as $C_{17}H_{14}O_7$ on the basis of its HREIMS and ^{13}C-NMR analysis Table 1. Its IR (ν_{max} 3400 (OH), 1647 (conj. carbonyl)) and UV (λ_{max}: 250, 320 and 320 nm) spectral data were consistent with a flavonol skeleton (Mabry, Markham, & Thomas, 1970). Its ^{1}H-NMR Table 1 revealed the presence of three phenolic protons (δ 9.20, 3H exchangeable with D_2O), two methoxyl groups (δ 3.85, (s, 6H)) and additional signals corresponding to an ABX spin system of three aromatic protons at δ 7.92, 7.02 and 6.02 ppm with *ortho* (J=8.7 Hz), *meta* (J=2.2 Hz) and *ortho–meta* (J=8.7, 2.2 Hz) coupling constants and two equivalent aromatic protons appearing as a 2H singlet at δ 7.52 ppm. The location of the two methoxyl groups on ring B at positions C-3′ and C-5′ was established from ion peaks at m/z 137 and 193 resulting from usual RDA fragmentation pattern in the EIMS of **2** as well as by ^{1}H NMR data and biogenetic grounds (Ebel, & Halhbrock, 1982). Hence, laurentinol (**2**) must be 3,7,4′-trihydroxy-3′,5′-dimethoxyflavone.

Compounds **3** and **4** are known isoflavones which were identified, in turn, as calycosin and gliricidin by comparison of their spectroscopic data with the published values (Malik, Sharma, & Seshadri, 1969; Manners, & Jurd, 1979). Isolated initially from

Table 1
^{1}H (300 MHz)–^{13}C (75.47 MHz) spectral data for laurentiquinone (**1**) = $CDCl_3$; and laurentinol (**2**) = DMSO-d_6; and ^{13}C-NMR (75.47 MHz) spectral data for calycosin (**3**) = DMSO-d_6; and gliricidin (**4**) = DMSO-d_6

Carbon	Laurentiquinone (**1**)		Laurentinol (**2**)		Calycosin (**3**)	Gliricidin (**4**)
	δC, m	δH (m, *J*, Hz)	δC, m	δH (m, *J*, Hz)	δC, m	δC, m
2	67.7 t	4.20 (m)	144.7 s	–	153.0 d	152.2 d
		4.45 (m)	–	–	–	–
3	30.9 d	3.60 (m)	138.9 s	–	123.3	123.6
4	29.2 t	2.63 (m)	172.0 s	–	174.5 s	174.5 s
4a	114.7 s	–	114.2[a] s	–	116.2 s	116.6 s
5	124.9 d	6.65 (d, 8.3)	126.4 d	7.92 (d, 8.5)	127.3 d	127.3 d
6	107.2 d	6.50 (d, 8.3)	114.7[a] d	6.92 (dd, 8.7 and 2.2)	115.1[a] d	115.1 d
7	155.6[a]	–	162.3 s	–	162.5 s	162.5 s
8	147.5 s	–	102.2 d	7.02 (d, 2.2)	102.2 d	102.1 d
8a	155.6[a] s	–	156.3 s	–	157.3 s	157.3 s
1′	134.9 s	–	121.4 s	–	124.7 s	127.2 s
2′	156.3[a] s	–	105.6 d	7.52 (s)	116.4[a] d	108.3[a] d
3′	178.0 s	–	147.8 s		146.0[a] s	150.3 s
4′	157.2[a] s	–	137.5 s		147.5[a] s	135.4 s
5′	107.3 d	–	147.8 s		111.9 d	150.3 s
6′	186.5 s	–	105.6 d	7.52 (s)	119.7 d	108.8[a] d
7-OH	–	5.60 (s)	–		–	–
8-OMe	67.4[a] q	3.96 (s)	–		–	–
2′-OMe	60.9[a] q	3.91 (s)	–		–	–
3′-OMe	–	–	56.2 q	3.85 (s)	–	–
4′-OMe	56.4[a] q	3.80 (s)	–	–	55.6 s	56.7 s
5′-OMe	–	–	56.2 q	3.85 (s)		

[a] Assignments may be reversed within the same column.

Millettia dielsiana (Malik et al., 1969) and *Gliricida sepium* (Manners, & Jurd, 1979), respectively, their ^{13}C NMR spectral data Table 1, reported here for the first time, were assigned on the basis of J_{Mod} and DEPT techniques as well as by comparison of these data with those of related compounds (Agrawal, 1989).

3. Experimental

3.1. General experimental procedures

All melting points were determined on a Klofler hot-stage apparatus and are uncorrected. ^{1}H and ^{13}C-NMR were recorded at 300.13 and 75.47 MHz, respectively, with TMS as internal standard. Mass spectra were obtained with an AVG analytical ZAB-HF mass spectrometer. Silica gel GF_{254} Merck and Silica gel 60 (70–230 mesh) were used for TLC and column chromatography, respectively. Alumin (activity 1) was also used for column chromatography.

3.2. Plant material

The heartwood of *Millettia* (Leguminosae) was collected at Ambam, Cameroon in August 1988. A voucher specimen documenting the collection is deposited at the National Herbarium, Yaounde, Cameroon.

3.3. Extraction and isolation

The dried, ground heartwood of *Millettia laurentii* (7.0 kg) was extracted successively at room temp. with *n*-hexane, chloroform and methanol. The evaporation of the various solvents yielded 75 g of hexane extract, 70 g of $CHCl_3$ and 200 g of MeOH extract.

The $CHCl_3$ extract was subjected to CC over Si gel and eluted with varying proportions of a mixture of hexane–EtOAc. A total of 125 fractions of ca. 250 ml each, were collected and combined on the basis of TLC analysis leading to five series (A–E). Series E, fractions 99–125, eluted with a mixture of hexane–EtOAc (1:1), was evaporated and the residue obtained (10 g) was subjected to repeated CC over Si gel eluted with a mixture of hexane–EtOAc of increasing polarity to afford laurentinol (**2**) 50 mg.

An aliquot portion (100 g) of MeOH extract was subjected to CC on alumina gel (activity 1) packed in CH_2Cl_2 and eluted with CH_2Cl_2 followed by a mixture of EtOAc–MeOH. A total 100 fractions of ca. 300 ml each, were collected and combined on the basis of TLC analysis leading to three series (F–H). Series F, fractions 49–63, eluted with EtOAc–MeOH (19:1), was further subjected to CC over alumina gel eluted with CH_2Cl_2, to give laurentiquinone (**1**) (25 mg). Series G was rechromatographed over alumina (activity) with a mixture of EtOAc–MeOH (9:1) to yield calycosin (**3**)

(15 mg) and gliricidin (**4**) (17 mg) after recrystallisation from acetone.

3.4. Laurentiquinone (*1*)

Orange prisms (acetone), m.p. 205–207°C, UV λ_{max} (MeOH) nm (log ε): 266 (4.10), 385 (3.06); IR (KBr) ν_{max}: 3500, 2920, 1680, 1650, 1100 and 800 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75.47 MHz), see Table 1; EIMS *m/z* (rel. int.%) 346 [M^+] (84), 331 (19), 299 (9), 285 (3), 271 (4), 256 (5), 243 (7), 231 (5), 207 (8), 194 (100), 179 (35), 165 (19), 151 (30), 133 (17), 123 (12), 109 (15), 69 (66) and 28 (31), HREIMS *m/z* 3346.3101 (calcd. for $C_{18}H_{18}O_7$ 346.3399).

3.5. Laurentinol (*2*)

Green prisms (MeOH) from hexane–EtOAc, m.p. 258–260°C; UV λ_{max} (EtOH) nm (log ε): 250 (4.05), 320 (2.93), 360 (4.23); IR (KBr) ν_{max}: 3400, 1647, 1540 and 1100 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) and ^{13}C-NMR (DMSO-d_6, 75.47 MHz), see Table 1; EIMS *m/z* (rel. int.%) 330 [M^+] (100), 315 (4), 301 (5), 284 (10), 198 (4), 193 (18), 183 (30), 182 (35), 137 (40), 136 (41), 108 (41), 84 (4), 66 (45) and 44 (46), HREIMS *m/z* 330.0741 (calcd. for $C_{17}H_{14}O_7$, 330.0739).

3.6. Calycosin (*3*)

Colourless prisms (MeOH), m.p. 243–245°C (lit. (Malik et al., 1969) 244–246°C); UV, IR and ^{1}H-NMR matched well with the literature data (Malik et al., 1969); ^{13}C-NMR (DMSO, 75.47 MHz-d_6) see Table 1; HREIMS *m/z* 284.06844 (calcd. for $C_{16}H_{12}O_5$, 284.06846).

3.7. Gliricidin (*4*)

Green prisms from hexane–EtOAc, m.p. 297–299°C (lit. (Manners, & Jurd, 1979) 298°); UV, IR and ^{1}H-NMR matched well with the literature data (Manners, & Jurd, 1979); ^{13}C-NMR (DMSO, 75.47 MHz-d_6), see Table 1; HREIMS *m/z* 300.06333 (calcd. for $C_{16}H_{12}O_6$, 300.06336).

Acknowledgements

We gratefully acknowledge the practical help of Dr. F. T. Mbenkum, Director of Environment at the Ministry of Environment and Forests in Cameroon with regards to the collection of the plant material. We also thank Dr. M. S. Tempesta for spectroscopic analyses.

References

Agrawal, P. K. (Ed.) (1989). In *Carbon-13 NMR of flavonoids* (ch. 1 and 3, p. 13 and p. 152). Amsterdam: Elsevier.

Ebel, E., & Halhbrock, K. (1982). In J. B. Harborne, & T. J. , eds). Mabry, *The flavonoids advances in research (* (p. 649). London: Chapman & Hall.

Fuendjiep, V., Nkengfack, A. E., Fomum, Z. T., Sondengam, B. L., & Bodo, B. (1998a). *Journal of Natural Products*, *61*, 380–383.

Fuendjiep, V., Nkengfack, A. E., Fomum, Z. T., Sondengam, B. L., & Bodo, B. (1998b). *Phytochemistry*, *47*, 113–115.

Kamnaing, P., Fanso Free, S. N. Y., Fomum, Z. T., Martin, M. T., & Bodo, B. (1994). *Phytochemistry*, *36*, 1561–1562.

Kurosawa, K., Ollis, W. D., Sutherland, O. I., Gottlieb, O. R., & Oliveira, D. A. B. (1978). *Phytochemistry*, *17*, 1405–1411.

Mabry, T. J., Markham, K. R., & Thomas, M. D. (1970). *The systematic identification of flavonoids*. Berlin: Springer.

Malik, S. B., Sharma, P., & Seshadri, T. R. (1969). *Indian Journal of Chemistry*, *7*, 118.

Manners, G., & Jurd, L. (1979). *Phytochemistry*, *18*, 1037.

Mbafor, J. T., Atchade, A. T., Nkengfack, A. E., Fomum, Z. T., & Olov, S. (1995). *Phytochemistry*, *40*, 949.

Mbenkum, T. F. (1986). *Systematic studies in genus Millettia* (ch. 2, pp. 45–50). Ph.D. thesis, University of Reading, UK.

Ngamga, D., Fanso Free, S. N. Y., Fomum, Z. T., Chiaroni, A., Riche, C., Martin, M. T., & Bodo, B. (1993). *Journal of Natural Products*, *56*, 2126–2132.

Ngamga, D., Fanso Free, S. N. Y., Fomum, Z. T., Martin, M. T., & Bodo, B. (1994). *Journal of Natural Products*, *57*, 1022–1024.

Sibata, H., & Sumio, S. (1978). *Heterocycles*, *10*, 85–86.

Singhal, A. K., Sharma, R. P., Baruah, J. N., Govindam, S. V., & Herz, W. (1982). *Phytochemistry*, *21*, 949–952.

Teesdale, C. (1954). *East African Medicinal Journal*, *31*, 301.