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CONRAUINONES C AND D, TWO ISOFLAVONES FROM STEM BARK OF *MILLETTIA CONRAUI**

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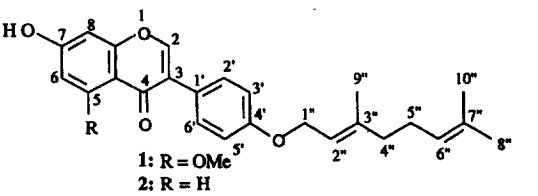
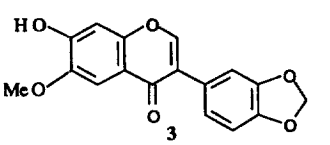
Key Word Index—*Millettia conraui*; Leguminosae; stem bark; isoflavones; conrauinones C and D; *O*-geranylation.

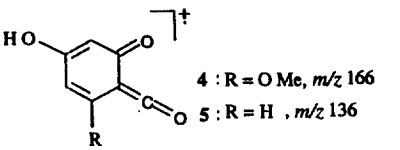
Abstract—Analysis of the stem bark of *Millettia conraui* led to the isolation of two new *O*-geranylated isoflavones named conrauinones C and D together with the known 7-hydroxy-6-methoxy-3′,4′-methylenedioxyisoflavone. The structure elucidation of the two new compounds by spectroscopic studies is described.

INTRODUCTION

The plant genus *Millettia* (Leguminosae) is well known for elaborating flavonoids [1], alkaloids [2] and diterpenoids [3] with insecticidal, piscicidal and molluscicidal activities [4, 5]. We have recently undertaken the investigation of Cameroonian plant *Millettia conraui* from which, we have isolated a wide range of flavonoids and isoflavonoids including conrauinones A and B [6, 7]. Further study of the isoflavonoids of this species resulted in the isolation of two new compounds, named conrauinones C (**1**) and D (**2**), along with the known 7-hydroxy-6-methoxy-3′,4′-methylenedioxy isoflavone (**3**).







RESULTS AND DISCUSSION

Air dried and powdered stem bark of *Millettia conraui* was extracted with benzene in a Soxhlet apparatus and the extract concentrated to dryness. The residue was then subjected to flash vacuum liquid chromatography over TLC grade silica gel followed by column chromatography to yield the three pure compounds (**1–3**). Compound (**3**) was identified as 7-hydroxy-6-methoxy-3′,4′-methylenedioxyisoflavone by comparison of its physical and spectral data with published values [8].

Conrauinone C (**1**), mp 178–180°, was obtained as colourless granules and reacted positively to the $FeCl_3$ reagent. The IR spectrum showed absorption bands at ν3464 and 1647 cm^{-1}, indicative of hydroxyl and carbonyl functions, respectively. The high resolution-EI mass spectrum showed a $[M]^+$ peak corresponding to the molecular formula $C_{26}H_{28}O_5$. A purple coloration of (**1**) in the Shinoda test, its UV spectrum [λ_{max}^{MeOH} nm: 248, 298] and ^1H NMR (δ 7.75 ppm for H-2) showed this compound to be an isoflavone. The ^1H NMR spectrum (Table 1) also revealed notable features including a set of signals consisted of three methyl singlets (δ 1.58, 1.62, 1.72), three methylene resonances (δ 2.07, 2.10, 4.53 ppm) and two olefinic protons (δ 5.14, 5.48 ppm) assignable to either neryl

* Part 9 in the series 'The Millettia of Cameroon'.

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Table 1. ^{1}H NMR (300.0 MHz, $CDCl_3$, δ ppm) and ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm) spectral data for conrauinones C (**1**) and D (**2**)

	Conrauinone C (**1**)		Conrauinone D (**2**)	
C	^{1}H [multiplicity, J (Hz)]	^{13}C, m	^{1}H [multiplicity, J (Hz)]	^{13}C, m
2	7.75 (*s*)	1.53.3 *d*	7.89 (*s*)	153.3 *s*
3	—	124.7 *s*	—	125.7 *s*
4	—	175.0 *s*	—	175.6 *s*
4a	—	117.4 *s*	—	118.6 *s*
5	—	151.4 *s*	8.15 (*d*, 8.8)	128.5 *d*
6	6.34 (*d*, 2.2)	118.6 *d*	6.90 (*dd*, 8.8, 2.2)	115.1 *d*
7	—	163.6[a] *s*	—	163.2[a] *s*
8	6.43 (*d*, 2.2)	103.7 *d*	6.83 (*d*, 2.2)	103.2 *d*
8a	—	159.2[a] *s*	—	158.8[a] *s*
1′	—	125.4 *s*	—	125.4 *s*
2′	7.43 (*m*)	115.6 *d*	7.45 (*m*)	115.6 *d*
3′	6.92 (*m*)	115.1 *d*	6.95 (2m)	115.1 *d*
4′	—	159.7[a] *s*	—	159.7[a] *s*
5′	6.92 (*m*)	115.1[b] *d*	6.95 (*m*)	115.1[b] *d*
6′	7.43 (*m*)	115.6[b] *d*	7.45 (*m*)	115.6[b] *d*
1″	4.53 (*d*, 6.5)	66.3 *t*	4.54 (*d*, 6.5)	66.3 *t*
2″	5.48 (*m*)	122.0 *d*	5.48 (*m*)	122.0 *d*
3″	—	140.9 *s*	—	140.9 *s*
4″	2.07 (*m*)	40.2 *t*	2.07 (*m*)	40.1 *t*
5″	2.10 (*m*)	27.0 *t*	2.15 (*m*)	27.0 *t*
6″	5.14 (*t*, 6.0)	118.4 *d*	5.08 (*t*, 6.0)	119.1 *d*
7″	—	132.1 *s*	—	132.1 *s*
8″	1.62 (*s*)	25.8 *q*	1.66 (*s*)	25.8 *q*
9″	1.72 (*s*)	16.7 *q*	1.72 (*s*)	16.6 *q*
10″	1.58 (*m*)	17.7 *q*	1.59 (*m*)	17.7 *q*
5-OMe	3.85 (*s*)	62.1 *q*	—	—

[a,b] assignments may be reversed within the same column.

or geranyl moiety which can be located on either the oxygen atoms at C-7 or C-4′ position. It has been shown by Kozawa *et al.* [9] that ^{13}C NMR data, particularly, the chemical shifts of the methyl at C-3″ and the methylene at C-4″ positions, aid in distinguishing a geranyl from neryl side chain. The chemical shifts at δ 16.7 and 40.2 ppm (Table 1) observed for methyl and methylene groups, respectively, confirm the presence of geranyl side chain in **1**. The ^{1}H NMR also showed one 3H singlet at δ 3.85 ppm corresponding to a methoxyl group, a pair of *meta* coupled protons at δ 6.34 and 6.43 ppm with $J = 2.2$ Hz due to H-6 and H-8 aromatic A ring protons. Moreover, a typical A_2B_2 spin system centred at δ 6.92 and 7.43 ppm established the presence of four aromatic protons H-2′, H-6′ and H-3′, H-5′, respectively, in the *para*-substituted B ring.

The lack of a chelated 5-hydroxyl signal in ^{1}H NMR between δ 11–13 ppm led us to locate the methoxyl group at C-5 position on ring A. This was confirmed by mass spectral fragmentation pattern which revealed an RDA ion fragment at m/z 166 (**4**) clearly indicating the placement of the methoxyl group on ring A at C-5 position. On the other hand, the second RDA ion fragment at m/z 254 resulting from B ring suggested clearly that the geranyl moiety is attached on the oxygen at C-4′ position. The placement of the geranyl side chain on the oxygen at C-4′ was further confirmed by 2D phase sensitive NOESY experiment which revealed interactions between methylene protons at C-1″ and B aromatic protons H-3′ and H-5′. The structure of conrauinone **1** is therefore, 7-hydroxy-5-methoxy-4′-*O*-[(*E*)-3,7-dimethyl-2,6-octadienyl] isoflavone.

Compound (**2**), colourless prisms, mp 188–190° and to which trivial name conrauinone D was assigned, was analysed for $C_{25}H_{26}O_4$ by high resolution mass spectrometry and gave the ^{1}H NMR spectrum of an isoflavone. In addition, it revealed the presence of unsubstituted C-5, C-6, C-8, a simple *para*-substituted B-ring and a geranyl substituent. In fact, when compared with data for **1**, the ^{1}H NMR and ^{13}C NMR spectral data of **2** showed a close similarity. The only major difference in the ^{1}H NMR of conrauinone D **2**, when compared with that of **1**, was that the doublet at δ 8.15 (1H, J 8.8 Hz) due to H-5 replaced the 3H singlet corresponding to the methoxyl substituent. These observations were sustained by the EIMS which revealed fragments for ring a unsubstituted at m/z 136 (**5**) and ring B with the geranyl substituent at m/z 254 allowing formulation of **2**, namely 7-hydroxyl-*O*-4′-[(E)-3,7-dimethyl-2,6-octadienyl] isoflavone.

EXPERIMENTAL

Plant material. Stem bark of *Millettia conraui* Harms was collected in May 1992 at Kumbo, North-West province of Cameroon. A voucher specimen documenting the collection was identified at the National Herbarium, Yaounde, Cameroon and is on deposit there.

Extraction and isolation. Air-dried powdered stem bark of *Millettia conraui* (4 kg) was extracted successively with C_6H_6 and MeOH. The C_6H_6 extract was filtered and evapd under red. pres. to give a viscous mass (114 g) of C_6H_6 extract. A part of this material (40 g) was subjected to flash chromatography on TLC grade silica gel eluted with C_6H_6–EtOAc mixt. A total of 50 frs of *ca* 300 ml each were collected and combined on the basis of TLC analysis leading to five main series (A–E). Frs 1–10 eluted with C_6H_6–EtOAc (9:1) gave series A (5.3 g). This series was rechromatographed over silica gel CC (70–230 mesh, ASTM; Merck). Initial elution with C_6H_6 and then with C_6H_6–EtOAc gradient afforded 7-hydroxy-6-methoxy-3′,4′-methylenedioxyisoflavone [**3**] (1 g). Frs 11–22 eluted with C_6H_6–EtOAc (8:2) gave series B. This series was further subjected to repeated CC over silica gel eluted with a mixt. of C_6H_6–EtOAc (17:3) to give conrauinone C [**1**] (300 mg). Series C, resulted from the combination of frs 23–32 eluted with the mixt. of C_6H_6–EtOAc (7:3), was rechromatographed on silica gel CC. The elution of this column with C_6H_6–EtOAc (4:1) yielded conrauinone D [**2**] (15 mg) after recrystallization in MeOH–EtOAc mixt.

Conrauinone C (**1**) Colourless granules, mp 178–180°; HRMS, *m/z*: 420.5098 (calcd for $C_{26}H_{28}O_5$: 420.5100); UV λ_{max}^{MeOH} nm (log ε): 248 (4.23), 298 sh (4.27); IR ν_{max} (KBr): 3464, 1647, 1510, 1465 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) see Table 1: ^{13}C NMR ($CDCl_3$, 75.45 MHz) see Table 1; EIMS *m/z* (rel. int. %): 420 $[M]^+$ (2), 312 (8), 284 (100), 255 (15), 254 (14), 238 (16), 225 (4), 190 (8), 166 (9), 149 (6), 137 (10), 118 (8), 93 (9), 84 (17), 69 (67).

Conrauinone D (**2**). Colourless, mp 188–190°; HRMS, *m/z*: 390.4830 (calcd for $C_{25}H_{26}O_4$: 390.4835); UV λ_{max}^{MeOH} nm (log ε): 247 (4.21), 298 sh (4.25); IR ν_{max} (KBr): 3465, 1648, 1610, 1510, 1465 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) see Table 1: ^{13}C NMR ($CDCl_3$, 75.45 MHz) see Table 1; EIMS, *m/z* (rel. int. %): 390 $[M]^+$ (2), 321 (8), 307 (100), 293 (15), 279 (5), 254 (100), 225 (4), 197 (8), 137 (10), 118 (8), 108 (5), 89 (9).

7-Hydroxy-6-methoxy-3′,4′-methylenedioxy-isoflavone (**3**). Colourless prisms, mp 260° (lit. [8] 260–262°); HRMS, *m/z* 312.2815 for $C_{17}H_{12}O_6$. IR, UV ^1H and ^{13}C NMR identical to published data [8].

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