Conrauinones A and B, Two New Isoflavones from Stem Bark of *Millettia conraui*[1]

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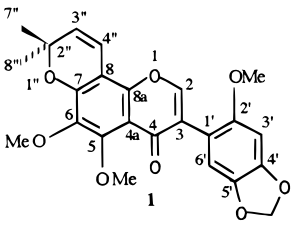
Two new isoflavones, named conrauinones A (**1**) and B (**2**), have been isolated from the stem bark of *Millettia conraui*, in addition to known 5-methoxydurmillone. The structures of the new compounds were determined by spectroscopic analysis including 2D NMR techniques as 5,6,2′-trimethoxy-4′,5′-(methylenedioxy)-2″,2″-dimethylpyrano[5″,6″:7,8]isoflavone (**1**) and 6-methoxy-3′,4′-(methylenedioxy)-7-*O*-[(*E*)-3″,7″-dimethyl-7″-ol-2″,5″-octadienyl]isoflavone (**2**).

The genus *Millettia* (Papilionaceae), represented by more than 200 species of climbers and trees, is distributed in tropical Africa, Asia, and Australia.[2] *Millettia conraui* Harms is a tree that grows in the undergrowth of the rainforest of Central and Northwest provinces of Cameroon.[3] Its use in the treatment of intestinal parasites and cholic in children, coupled with the fact that other members of this genus have been reported to show insecticidal, piscicidal, and molluscicidal activities,[4−5] prompted our investigation on this species.

Previous chemical investigations of a number of *Millettia* species have also shown them to be a good source of flavonoids, isoflavonoids, and nitrogenous compounds.[1,7−8] In the present paper, we report the isolation and structure elucidation of two new isoflavones, named conrauinones A (**1**) and B (**2**), from the stem bark of *M. conraui*, along with the known 5-methoxydurmillone.



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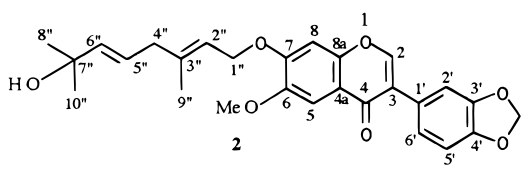

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Dried and ground stem bark of *Millettia conraui* was extracted with C_6H_6 and MeOH successively. Repeated Si gel column chromatography of the concentrated MeOH extract, by using gradient elution from C_6H_6 to EtOAc, afforded the three compounds **1**, **2**, and 5-methyldurmillone. The fact that all of these compounds reacted positively to the Shinoda test (purple coloration in the presence of Mg/HCl) suggested their flavonoid nature.[9]

Compound **1**, conrauinone A, was obtained as colorless prisms, mp 226−228 °C. The molecular ion $[M]^+$ at *m/z* 438.1312 in the HREIMS corresponds to the molecular formula $C_{24}H_{22}O_8$ (calcd 438.1315), indicating 14 double bond equivalents (DBE). The broad band decoupled ^{13}C-NMR spectrum of compound **1** showed 24 carbon signals. The analysis of this spectrum by the aid of J_{Mod} and DEPT techniques unequivocally indicated that five of these were methyls, one was a methylene, and five were methines. Hence, there were 13 quaternary carbons (among which were one carbonyl, 11 sp^2 carbons, and one sp^3 carbon). The UV spectrum (λ_{max} 295 nm, λ_{max} sh 325 nm) of conrauinone A was typical of compounds having an isoflavone skeleton.[10,11] This was supported by ^{1}H- and ^{13}C-NMR spectra, which showed resonance signals at δ 7.98 for H-2 and δ 152.1 ppm for C-2 (Table 1) characteristic of isoflavone.[10,12]

Also, the ^{1}H-NMR spectrum showed an AB spin system consisting of two doublets at δ 5.64 and 6.74 ppm (J = 10.0 Hz), which, together with the 6H singlet at δ 1.51 ppm, suggested the presence of 2″,2″-dimethylpyrano substituent. The base ion peak at *m/z* 423 $[M - Me]^+$ in the EIMS and the set of signals at δ 28.0, 78.1, 115.1, and 129.1 ppm in the ^{13}C-NMR spectrum provided further support for the presence of a *gem*-dimethylchromene system.[6] In the aromatic region, the appearance of two singlets at δ 6.57 and 6.79 ppm established the presence of two para-coupled aromatic protons on ring B. The ^{1}H NMR further revealed the presence of three methoxyl groups (δ 3.69, 3.86, and 3.96 ppm) and a methylenedioxy moiety at δ 5.91 ppm. From the above results, the nature of substituents attached to the

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Table 1. ^{13}C- (75 MHz) and ^{1}H- (300 MHz) NMR Data for Conrauinones A (1) and (2) in $CDCl_3$

carbon no.	conrauinone A (**1**)		conrauinone B(**2**)[a]	
	δ_C m	δ_H (mult, *J* Hz)	δ_C m	δ_H (mult, *J* Hz)
2	152.1 d	7.98 (s)	151.9 s	7.89 (s)
3	122.2 s		125.3 s	
4	175.0 s		175.4 s	
4a	113.2 s		117.7 s	
5	152.9 s		123.8 d	7.59 (s)
6	140.0 s		148.0 s	
7	150.9 s		153.6 s	
8	106.7 s		100.6 s	6.84 (s)
8a	149.1 s		152.1 s	
1′	112.8 s		125.9 s	
2′	153.0 s		109.8 d	7.09 (d, 1.7)
3′	95.3 d	6.57 (s)	147.7 s	
4′	148.3 s		147.6 s	
5′	141.1 s		108.4 d	6.85 (d, 8)
6′	111.4 d	6.79 (s)	122.3 d	6.96 (dd, 8.0, 1.7)
1″			66.3 t	4.70 (d, 6.5)
2″	78.1 s		119.2 d	5.54 (t, 6.5)
3″	129.1 s	5.64 (d, 10.0)	140.9 s	
4″	115.1 s	6.74 (d, 10.0)	42.2 t	2.77 (d, 5.3)
5″			123.9 d	5.65 (d, 15.5)
6″			140.4 d	5.60 (dt, 15.5, 6.5)
7″	28.0 q	1.51 (s)	70.7 s	
8″	28.0 q	1.51 (s)	30.9 q	1.29 (s)
9″			16.8 q	1.75 (s)
10″			29.8 q	1.29 (s)
5-OMe	62.2[b] q	3.96[b](s)		
6-OMe	61.4[b] q	3.86[b](s)	56.3 q	3.95 (s)
2′-OMe	56.8 q	3.69 (s)		
OCH_2O	101.3 t	5.91 (s)	101.1 t	5.97 (s)

[a] δ7″-OH: 1.48 (1H, s, exchangeable in D_2O). [b] Assignments may be interchanged within the same column.

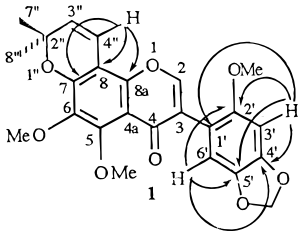

Figure 1. Selected HMBC correlations for conrauinone A (**1**)

isoflavone nucleus was established. Their positions were elucidated as follows.

In the ^{13}C-NMR spectrum, the occurrence of all quaternary oxygenated sp^2 carbons in the range of 140−150 ppm showed the presence of oxygen on adjacent carbons[12] (viz., C-4′ and C-5′, and C-6 and C-7). The placement of the substituents on the A and B rings derived from the mass spectral fragmentation pattern, which exhibited fragment ions at *m*/*z* 176 (B_1)[10,13] and 247 caused by the usual retro-Diels−Alder (RDA) cleavage from ion $[M - Me]^+$ at *m*/*z* 423. The ion peak at *m*/*z* 176 can be assigned to ring B carrying one methoxyl group and the methylenedioxy substituent. The relative positions of these groups on ring B were determined on the basis of the HMBC spectrum (Figure 1), which showed, through ^2J and ^3J interactions, that the para-coupled aromatic protons at δ 6.79 and δ 6.57 were correlated to C-2′ (δ 153.0), C-4′ (δ 148.3), and C-5′ (δ 141.1); thus, these protons were assigned to H-6′ and H-3′, respectively, leading to the location of the methoxyl group at C-2′ and the methylenedioxy group at C-4′, C-5′.

On the other hand, the ion peak at *m*/*z* 247 in the EIMS clearly indicated that compound **1** possesses two

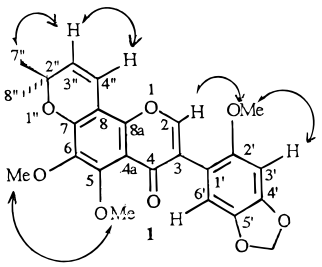

Figure 2. Selected NOESY correlations for conrauinone A (**1**)

methoxyl and the 2,2-dimethylpyran substituents on ring A. It remained to establish unambiguously whether the fusion of the 2,2-dimethylpyran moiety on ring A was linear or angular. This was deduced from 2D NOESY spectrum (Figure 2), which showed no correlations between methoxyl protons and vinylic protons at δ 6.74, but correlation contours between methoxyl protons at δ 3.96 and δ 3.86. This finding indicated clearly that the *gem*-dimethylpyran moiety was fused in an angular manner on ring A. Thus, one methoxyl group was located at C-5 and the second one at C-6. This was further supported by the HMBC spectrum (Figure 1) in which the signal at δ 6.74 (H-4″) was observed to have correlations with C-7 (δ 150.9), C-8 (δ 106.7), and C-8a (δ 149.1) but no signs of correlation with C-5 (δ 152.9). From the above spectroscopic studies, conrauinone A (**1**) was deduced to be 5,6,2′-trimethoxy-4′,5′-(methylenedioxy)-2″,2″-dimethylpyran-[5″,6″:7,8]isoflavone.

Compound **2**, to which we have assigned the trivial name conrauinone B, was obtained as colorless prisms, mp 246 °C. It was analyzed for $C_{27}H_{28}O_7$ by HREIMS, which showed a molecular ion peak at *m*/*z* 464.51998 (calcd 464.520 01). Again, the UV (λ_{max} 298 nm),[10,11] the ^1H-NMR (δ 7.94 for H-2), and the ^{13}C-NMR (δ 151.9 for C-2) spectra (Table 1) showed conrauinone B (**2**) to be an isoflavone derivative.[10,12] The ^1H-NMR spectrum of **2** also displayed two sharp 1H singlets at δ 7.59 and 6.84 assignable to two para-coupled aromatic protons H-5 and H-8 of ring A, a 3H singlet at δ 3.95 due to one methoxyl group and a sharp 2H singlet at δ 5.97 ppm corresponding to methylenedioxy substituent. Furthermore, the ^1H NMR also exhibited a typical ABX spin system at δ 7.09 (1H, d, *J* = 1.74 Hz, H-2′), 6.96 (1H, dd, *J* = 8.0 Hz and 1.7, H-6′), and 6.85 (1H, d, *J* = 8.0 Hz, H-5′), which consisted of three aromatic protons on ring B with *meta*, *ortho*/*meta*, and *ortho* coupling, respectively. The side-chain moiety was also shown by ^{13}C-NMR (Table 1) and DEPT data analysis to contain 10 carbons, including two quaternary carbons, three methyls, two methylenes, three olefinic methines, and, hence, two quaternary carbons. This was confirmed by the presence in the ^1H-NMR spectrum of a set of signals corresponding to three methyl groups of which two appear at δ 1.29 as a singlet and the third one at δ 1.75; two methylene as doublets at δ 4.70 (2H, *J* = 6.5) for H-13 and δ 2.77 (*J* = 5.3) for H-4″; three olefinic protons centered at δ 5.54 (t), 5.63 (m), and 5.60 (dt), and a D_2O exchangeable hydroxyl signal at δ 1.48, suggesting the presence of either geraniol or nerol moiety. It has been shown by Kozawa et al.[14] that ^{13}C-NMR data, particularly the chemical shift of the methyl at the C-3″ position and the methylene at C-4″, aid to distinguish

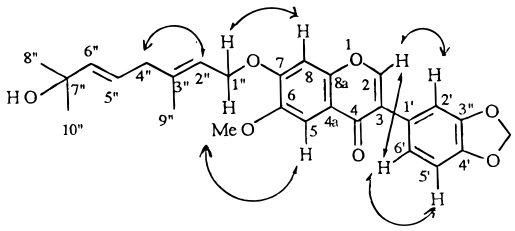

Figure 3. Selected NOESY interactions for conrauinone B (**2**)

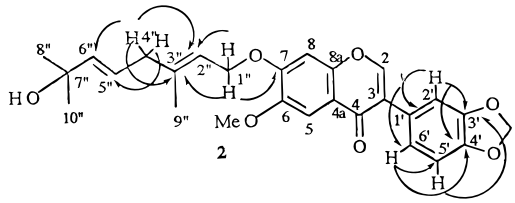

Figure 4. Selected HMBC correlations for conrauinone B (**2**)

a geranyl from a neryl side chain. The chemical shift at δ 16.8 and 42.2 ppm observed for methyl and methylene, respectively, confirmed the presence of a geraniol side chain in compound **2**. The location of geranyl, methoxyl, and methylenedioxy substituents was derived from the mass spectral fragmentation pattern that revealed important ion fragments at *m*/*z* 312, 166, and 146 (B_1).[10,13] The fragmentation ions at *m*/*z* 146 (B_1) and 166, resulting from RDA cleavage of ion $[M - 152]^+$, showed clearly the location of the methoxyl and geraniol substituents on ring A and the methylenedioxy substituent on ring B, respectively. Therefore, it remained to establish unambiguously their positions on that ring. The interactions observed in the phase-sensitive NOESY (Figure 3) experiment between methylene protons at C-1″ and H-8 aromatic proton on the one hand and between protons of methoxyl group and H-5 aromatic proton on the other hand, permitted us to locate the geraniol moiety on the oxygen atom at the C-7 position and the methyl group on the oxygen at the C-6 position. This was further confirmed by an HMBC experiment showing long-range correlation between protons H-1″ and the carbon signals at δ 140.9 (C-3″) and 153.6 (C-7) (Figure 4). In addition, the proton signal at δ 3.95 ppm was correlated with a carbon signal at δ 148.0 ppm (C-6). On the basis of the above spectroscopic studies, conrauinone B (**2**) was thus identified as 6-methoxy-3′,4′-(methylenedioxy)-7-*O*-[(*E*)-3″,7″-dimethyl-7″-ol-2″,5″-octadienyl]isoflavone.

5-methoxydurmillone, obtained as colorless prisms, had an empirical formula of $C_{23}H_{20}O_7$. NMR data (^{1}H, ^{13}C, HETCOR, NOESY) were identical to the same compound isolated from the stem bark of *Millettia ferruginea*.[6]

Experimental Section

General Experimental Procedures. All melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin−Elmer 727B spectrometer in KBr disks. UV spectra were obtained on a Beckman model 25 spectrophotometer. EIMS (ionization voltage, 70 eV) were measured with LKB9000S and Nermag/Sidar U 3:1 spectrometers. ^{1}H- and ^{13}C-NMR spectra were taken on a Bruker spectrometer equipped with a 5-mm ^{1}H and ^{13}C probe operating at 300 and 75 MHz, respectively, with TMS as internal standard. DEPT, J_{Mod}, and 2D NMR spectra (HMQC, HMBC, and NOESY) were measured with the usual pulse sequence, and data processing was obtained with standard software.

Plant Material. Stem bark of *M. conraui* Harms was collected in May 1992 at Kumbo, Northwest 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 *M. conraui* (4 kg) was extracted successively with C_6H_6 and MeOH. The MeOH extract was filtered and evaporated on a rotatory evaporator under reduced pressure to obtain a viscous mass (176 g) of MeOH extract. This material was subjected to column chromatography on Si gel 60 (70−230 mesh, ASTM; Merck) packed in C_6H_6 and eluted with C_6H_6−EtOAc mixture. A total of 250 fractions of ca. 250 mL each were collected and combined on the basis of TLC analysis leading to six main series (A−F). Fractions 1−50 eluted with C_6H_6−EtOAc (9:1) gave series A (4 g). This series was rechromatographed with Si gel column chromatography. Initial elution with C_6H_6 and then with C_6H_6−EtOAc gradient afforded 5-methoxydurmillone (3 g). Fractions 51−120 eluted with C_6H_6−EtOAc (8:2) gave series B. This series was further subjected to repeated column chromatography over Si gel eluted with a mixture of C_6H_6−EtOAc (17:3) to give conrauinone A (**1**) (300 mg). Series E, resulting from the combination of fractions 150−200 eluted with the mixture of C_6H_6−EtOAc (1:1), was rechromatographed with Si gel column chromatography. The elution of this column with EtOAc−MeOH (19:1) yielded conrauinone B (**2**) (15 mg) after recrystallization with MeOH.

Conrauinone A (1): colorless prisms (acetone) mp 226−228 °C; UV (MeOH) λ_{max} (log ϵ) 224 (4.41), 263 (4.54), 295 (sh) (4.17), 320 (sh) (3.80) nm; IR υ_{max} 1660, 1631, 1535, 1510, 1425, 1420, 1375, 1362, 1290, 1180 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; EIMS *m*/*z* 438 $[M]^+$ (32), 423 (100), 407 (28), 393 (10), 377 (6), 363 (11), 349 (5), 279 (3), 247 (18), 189 (7), 176 (17), 161 (8), 149 (6); HREIMS *m*/*z* 438.1312 (calcd for $C_{24}H_{22}O_8$, 438.1315).

Conrauinone B (2): colorless prisms (MeOH) mp 246 °C; UV (MeOH) λ_{max} (log ϵ) 247 (4.23), 298 (sh) (4.27) nm; IR υ_{max} 1660, 1631, 1535, 1510, 1425, 1420, 1375, 1362, 1290, 1180 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; EIMS *m*/*z* 464 $[M]^+$ (5), 337 (93), 313 (28), 312 (100), 298 (4), 281 (7), 268 (5), 253 (3), 166 (8), 152 (9), 146 (20), 145 (8); HREIMS *m*/*z* 464.519 98 (calcd for $C_{27}H_{28}O_7$, 464.520 01).

5-methoxydurmillone: colorless prisms (C_6H_6): mp 140−142 °C (lit.[6] 142−143 °C); HREIMS *m*/*z* 408.1223 (calcd for $C_{23}H_{20}O_7$, 408.1209).

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