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Phytochemistry 64 (2003) 599–602
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Three isoflavanones with cannabinoid-like moieties from *Desmodium canum*

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Received 16 January 2003; received in revised form 12 March 2003
Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Three further derivatives of 5,7,2′,4′-tetrahydroxy-6-methyl isoflavanone have been isolated from the root extract of *Desmodium canum* and assigned the structures 2,3-dihydro-5,7-dihydroxy-6-methyl-3-(1a,2,3,3a,8b,8c-hexahydro-6-hydroxy-1,1,3a-trimethyl-1H-4-oxabenzo[f]cyclobut[c,d]inden-7-yl)-4H-1-benzopyran-4-one (**1**) 2,3-dihydro-5,7-dihydroxy-6-methyl-3-(6a,7,8,10a-tetrahydro-3-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2-yl)-4H-1-benzopyran-4-one (**2**) 2,3-dihydro-5,7-dihydroxy-6-methyl-3-(3-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2-yl) 4H-1-benzopyran-4-one (**3**). The three compounds and the previously isolated chromene **4** all derive from the geranylated precursor **5** by a series of cannabinoid-like oxidative rearrangements.

Keywords: Keywords; *Desmodium canum*; Leguminosae; Isoflavanones; Cannabinoid-like substitutions

1. Introduction

Some species of *Desmodium* (including *Desmodium canum*) are important for soil preservation and potentially available as forage, while others are used in folk medicine (Avasthi et al., 1955; Ghosal et al., 1972). The presence of C-glucosyl flavonoids is a common feature of *Desmodium* genus, whereas only an example of an isoflavonoid—a pterocarpan—can be found in the literature (Purushotaman et al., 1971).

In a previous paper we reported the isolation of three antimicrobial isoflavanones, namely desmodianone A, B, and C, from the CH_2Cl_2-soluble portion of the ethanolic extract of the roots of *D. canum* (Delle Monache et al., 1996). A re-examination of the fraction, containing the dihydropyrano isoflavanone **4** as major component, afforded three new minor compounds related to **4**. This paper deals with the structure elucidation of the three compounds.

2. Results and discussion

The three isoflavonoids **1**–**3** were isolated by extended column and preparative thin-layer chromatography of a mixture obtained during the purification of compound **4** (Delle Monache et al., 1996). Spectral data revealed the general features of an isoflavanone nucleus (Mabry et al., 1970; Markham and Chari, 1982): absorption bands at 292 and 320 nm (UV), an absorbance at 1625 cm^{-1} (IR), the doublets of doublets of an ABC system for 2H-2 and H-3 protons and the signals for C-2 (ca. δ 71), C-3 (δ 51) and C-4 (δ 198) carbons (^1H and ^{13}C NMR spectra, see Table 1). In particular, as in compound **4**, the highfield C-3 resonance values were correlated to the presence of a 2′-OH group; moreover, an aromatic methyl group (Table 1) was still present and located at C-6, based on the delay in the appearance of a bathochromic shift with $AlCl_3$ in the UV spectrum (Delle Monache et al., 1977) and the resonance values in the

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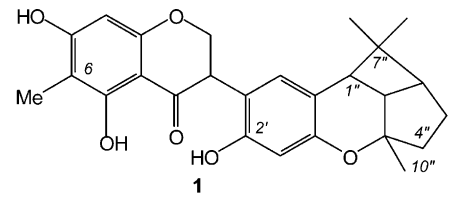
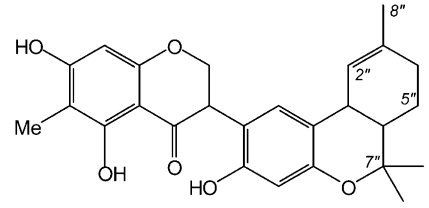
0031-9422/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00201-2

^{13}C NMR spectra (Table 1) of C-6 itself (Agrawal et al., 1981). The position of the methyl group was confirmed by HETCOR long-range experiments. Hence, the three compounds were shown to have a common 4*H*-1-5,7-dihydroxy-6-methylbenzopyran-4-one moiety.



1



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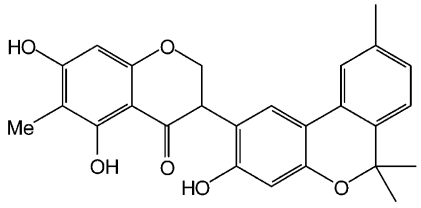

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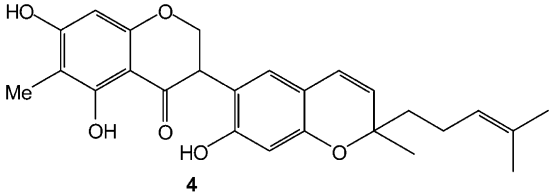
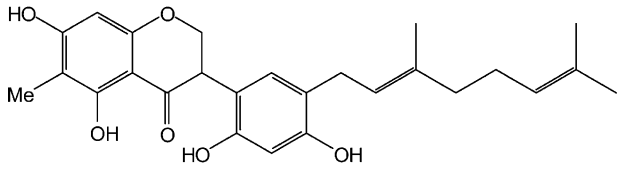


4



5

Whereas **1** and **2** gave in the mass spectrum a molecular peak at *m*/*z* 436, isomeric with that of **4**, compound **3** showed $[M]^+$ at *m*/*z* 432. On the basis of HR-EI–MS, the molecular formulae $C_{26}H_{28}O_6$ and $C_{26}H_{24}O_6$ were ascribed to the pair **1**/**2** and to **3**, respectively. Since H-3′ and H-6′ aromatic protons are still present (NMR spectra in Table 1), the three compounds differ in the substituents of C-4′ and C-5′ carbons. In the mass spectra of **1, 2** and **4** the peak at *m*/*z* 167, typical of the A ring of 6-methylisoflavanones, and the fragments at *m*/*z* 187, 213, and 253 (base peak), all related to the B ring, were always present; the last three ions account also for the presence of a 1-methylpyran ring substituted by different units in the three molecules (Delle Monache et al., 1977). The common molecular formula of **1**, **2** and **4** ($C_{26}H_{28}O_6$) requires that two double equivalent bonds are due to these moieties. In the ^{1}H NMR spectrum of compound **1** no signal for olefinic protons was present, requiring the unknown moiety to consist of two rings. The yet unassigned signals in ^{1}H and ^{13}C NMR spectra revealed that the two rings contain two methyls, two methylenes, three connected methines and two quaternary carbons. A plausible arrangment of these groups can be found in the cyclol unit, widely distributed in several natural products: cannabicyclol (Ganoi and Mechoulan, 1971), *o*-cannabicyclol (bibenzyl; Crombie et al., 1988), eriobrucinol (coumarin; Jefferies and Worth, 1973), bicyclomahaimbine (alkaloid; Kureel et al., 1969), clusiacyclol A (benzophenone; Gonzalez et al., 1995) and kuwanon D (Nomura and Fukai, 1981), which is a 5,7-dihydroxyflavanone with the same B ring as **1**. ^{1}H and ^{13}C NMR spectral data, as compared with those reported in the literature for the above compounds, assign to **1** the structure 2,3-dihydro-5,7-dihydroxy-6-methyl-3-(1a,2,3,3a,8b,8c-hexahydro-6-hydroxy-1,1,3a-trimethyl-1H-4-oxabenzo[f]cyclo- but [cd]inden-7-yl)-4H-1-benzopyran-4-one. For the sake of simplicity the compound was named desmodianone D. Molecular models, coupling constants and NOE difference experiments require the three methine protons and the 3a-methyl to be all *cis*.

In compound **2** a double bond and a ring must be present (NMR evidence). Examination of proton connectivities, by COSY, revealed the presence of a cyclohexene ring, with a methyl group on one of the sp^2 carbons, and *cis*-fused ($J_{\text{H-1}'',\text{H-2}''} = 5.5$ Hz) with the dihydropyran nucleus. The 6a,7,8,10a-tetrahydro-6,6,9-trimethyl-6H-benzo[b,d]pyran moiety was found in cannabinoids either with a *trans* (Ganoi and Mechoulan, 1971) or *cis* junction (Smith and Kempfert, 1977). After comparison with literature data, compound **2** was assigned the structure 2,3-dihydro-5,7-dihydroxy-6-methyl-3-(6a,7,8,10a-tetrahydro-3-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2-yl)-4H-1-benzopyran-4-one and named desmodianone E.

The third compound **3** ($[M]^+$ at *m*/*z* 432, four H less than the others) showed in NMR spectra the signals for a further aromatic ring, formally originated by dehydrogenation of the cyclohexene ring of **2**. This change is reflected in a different mass fragmentation, dominated by a base peak corresponding to an ion $[M-Me]^+$ typical of chromene (dihydropyran) derivatives. The structure 2,3-dihydro-5,7-dihydroxy-6-methyl-3-(3-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2-yl)-4H-1-benzopyran-4-one was thus assigned to compound **3**.

A search in the literature afforded, inter alia, a cannabinoid with the same three rings system (Ganoi and Mechoulan, 1971), but particularly an isoflavanone,

Table 1
^{1}H and ^{13}C NMR spectral data (δ) of isoflavanones from *Desmodium canum*

Position		**1**			**2**		**3**
2	69.72; 69.38	4.77; 4.71 *dd* (11.5, 5.5) 4.62; 4.61 *dd* (11,5, 4.8)	2	69.57; 69.50	4.80; 4.80 *dd* (11.5,5.5) 4.63; 4.61 *dd* (11,5,5)	69.51	4.88 *dd* (11.5, 5.3) 4.69 *dd* (11.5, 5)
3	45.39; 45.12	4.03; 3.99 *dd* (5.5, 4.8)	3	46.17; 45.25	4.00; 3.97 *dd* (5.5, 5)	45.48	4.06 *dd* (5.3, 5)
4	196.78; 196.75	–	4	197.13	–	196.66	–
5	162.00; 161.96	OH: 12.07 *s**	5	162.22; 162.*16*	OH: 12.07; 12.06 *s**	162.01	OH: 12.03 *s**
6	104.11; 104.01	–	6	104.19; 104.09	–	104.29	–
7	162.97	OH: 6.10 *s**	7	162.31; 163.04	OH: 6.10 *s**	163.2	OH: 6.05 *s**
8	94.55; 94.32	5.96; 5.95 *s*	8	94.60; 94.38	5.98; 5.96 *s*	94.69	6.01 *s*
9	160.31; 160.29	–	9	160.59; 160.53	–	160.33	–
10	101.54; 101.11	–	10	101.88; 101.69	–	101.61	–
1′	114.82; 114.41	–	1′	114.56; 114.47	–	115.59	–
2′	154.02; 154.06	OH: 7.15 *s**	2′	154.47; 153.25	OH: 6.95 *s**	155.62	OH: 7.55 *s**
3′	106.77; 106.70	6.44; 6.43 *s*	3′	105.58; 105.40	6.34; 6.31 *s*	106.53	6.51 *s*
4′	153.44; 153.41	–	4′	152.8	–	153.69	–
5′	117.44; 117.19	–	5′	117.90; 117.84	–	116.03	–
6′	128.46; 128.09	6.86; 6.83 *s*	6′	128.13; 127.13	7.22; 7.20 *s*	122.04	7.72 *s*
1″	39.25; 39.26	2.94; 2.93 *d* (8)	1″	31.56; 31.50	3.44; 3.92 *t* (5.5)	127.84	–
2″	39.18; 39.24	2.59; 2.56 *d* (9.5)	2″	39.28; 39.19	1.59; 1.55 *ddd* (12.5, 5.5, 2.5)	135.4	–
3″	83.91	–	3″	76.25; 76.20	–	77.92	–
4″	39.19; 37.60	1.85; 1.65 *m*	4″	121.84	5.86; 5.82 *dtq* (5.5, 1.5, 0.5)	121.71	7.37 *d* (1.7)
5″	25.34	1.65; 1.50 *m*	5″	134.98; 134.84	–	136.9	–
6″	46.38; 46.34	2.37; 2.35 *ddd* (8,7.5,1)	6″	30.29; 30.16	1.95 *m*	127.66	7.03 *dd* (7.8, 1.7)
7″	38.68; 38.62	–	7″	19.49; 19.46	1.85 (eq); 1.26 (ax)	122.86	7.07 *d* (7.8)
6-Me	6.64	2.00 *s*	6-Me	6.5	2.01	6.66	2.00 *s*
3″-Me	27.02; 26.72	1.34; 1.31	3″-Me	26.45	1.39	27.62	1.59 *s*
7″-Me	34.76; 34.67	1.32 *s*	3″-Me	26.26	1.25; 1.23	27.54	1.55 *s*
8″-Me	19.37; 19.15	0.67; 0.57 *s*	6″-Me	26.42; 23.34	1.70; 1.65	21.3	2.37 *s*

*All these signals disappear upon addition of D_2O.

tetrapterol A (Tanaka et al., 1994; Iinuma et al., 1995), which differs from **3** only in the absence of the 6-methyl group. Comparison of the spectral data confirmed the close similarity between the two molecules and for **3** we propose therefore the name 6-methyltetrapterol A.

The co-occurrence of the three compounds with **4** reflects a déjà vu situation for cannabis compounds. The bulky substituents of the B ring of *Desmodium* isoflavanones and of the aromatic ring of hashish components are produced by similar enzyme systems which catalyze the same rearrangements of a geranyl chain in two different frameworks. In the case of isoflavanones of *D. canum*, the precursor can be identified as desmodianone B (**5**), previously isolated from the same plant (Delle Monache et al., 1996).

An intriguing characteristic of isoflavanones **1** and **2** was the doubling of most of the signals in proton and carbon spectra. This feature could be attributed to the existence of two different stereoforms of the dihydropyran ring, but the differences in chemical shift would be much more dramatic. Moreover, we have seen that the ring junctions A′/B′ (in **2**) and A′/B′C′ (in **1**) are always *cis*. Finally, a conformational equilibrium for the dihydropyran ring was excluded when no significant change was introduced by running the spectra at higher temperature (even with DMSO-d_6 at 110 °C). Since the phenomenon has not been described for many other natural products containing the cyclol (as in **1**) and benzopyran (as in **2**) moieties, it must be ascribed to the simultaneous presence of the isoflavanone nucleus. In particular, we may suppose a partial epimerization at C-3, occurred in the three compounds during their purification. The positive Cotton effects, in the 330–350 range of the CD curves of **1**, **2** and **3**, require a 3*R*-configuration to be assigned to the major component of the two diastereoisomeric (**1** and **2**) and scalemic (**3**) mixtures.

3. Experimental

3.1. General

All the isolated compounds were vitreous solids. Spectroscopic measurements were conducted with the following instruments and media: IR, $CHCl_3$; UV, MeOH, EIMS, AEIMS 12, direct inlet; CD, JASCO J-500A, MeOH; NMR (^{1}H and ^{13}C), Varian XL 400, $CDCl_3$.

3.2. Plant material

Roots of *D. canum* (Gmal.) Schinz and Tellung (Leguminosae) were collected in Paulista (Pe, Brazil). A

voucher specimen (IA-5168) has been deposited in the Herbarium of Departamento de Antibioticos, Recife (Pe, Brazil).

3.3. Extraction and fractionation

Fraction DD-II (1.2 g) of the CH_2Cl_2 extract gave on silica gel with $CHCl_3$ the main component **4** (0.85 g), previously described (Delle Monache et al., 1996), and a mixture (0.1 g) of **1**, **2** and **3**. The three minor products were separated by a combination of CC and preparative TLC (silica gel, *n*-hexane/EtOAc mixtures).

3.4. Desmodianone D, *1*

18 mg: UV λ_{max} nm (log ε): 293 (4.30); (+ AcONa): 293*sh*, 330; (+ $AlCl_3$): 314, 363 (after 20′); CD ($c = 0.0001$) $[\Phi]_{240}$, $[\Phi]_{284}$ −852, $[\Phi]_{293}$ 0, $[\Phi]_{310}$ +639, $[\Phi]_{345}$ 0; IR ν_{max} cm^{-1}: 3595, 3250, 1630, 1600, 1490, 1295, 1155, 825; ^1H and ^{13}C NMR spectra in Table 1. HR-EI–MS: $[M]^+$ 436.1902 ($C_{26}H_{28}O_6$ requires 436.1886); EI–MS *m*/*z* (rel. int): 436 $[M]^+$ (22), 421 $[M-Me]^+$ (6), 418 $[M-H_2O]^+$ (3), 353 [**d**]$^+$ (100), 213 [**c**]$^+$ (38), 187 [**b**]$^+$ (19), 185 [213–CO]$^+$ (11), 167 [**a**]$^+$ (12), 149 [**a**–H_2O]+ (12); *m** 406 (436–421), 285.8 (436–353), 160.7 (213–185), 128.5 (353–213).

3.5. Desmodianone E, *2*

27 mg: UV λ_{max} nm (log ε): 293 (4.29); (+ AcONa): 294, 328; (+ $AlCl_3$): 314, 364 (after 20′); CD ($c = 0.0001$) $[\Phi]_{240}$ 0, $[\Phi]_{283}$ −452, $[\Phi]_{293}$ 0, $[\Phi]_{309}$ +465, $[\Phi]_{345}$ 0; IR ν_{max} cm^{-1}: 3595, 3250, 1630, 1600, 1490, 1295, 1155, 825; ^1H and ^{13}C NMR spectra in Table 1. HR-EI–MS: $[M]^+$ 436.1906 ($C_{26}H_{28}O_6$ requires 436.1886); EI–MS *m*/*z* (rel. int): 436 $[M]^+$ (75), 421 $[M-Me]^+$ (17), 418 $[M-H_2O]^+$ (11), 353 [**d**]$^+$ (100), 213 [**c**]$^+$ (33), 187 [**b**]$^+$ (47), 185 [213–CO]$^+$ (9), 167 [**a**]$^+$ (39), 149 [**a**–H_2O]$^+$ (10); *m** 406.5 (436–421), 285.8 (436–353), 160.7 (213–185), 128.5 (353–213).

3.6. 6-Methyltetrapterol A, *3*

18 mg: UV λ_{max} nm (log ε): 285sh (4.27), 293 (4.29) 325*sh* (4.0); (+ AcONa): 281, 293*sh*, 328; (+ $AlCl_3$): 279, 316, 362 (after 20′); CD ($c = 0.0001$) $[\Phi]_{240}$ 0, $[\Phi]_{283}$ −267, $[\Phi]_{293}$ 0, $[\Phi]_{309}$ +255, $[\Phi]_{345}$ 0; IR ν_{max} cm^{-1}: 3590, 3250, 1630, 1595, 1495, 1290, 1155, 820; ^1H and ^{13}C NMR spectra in Table 1. HR-EI–MS: $[M]^+$ 432.1568 ($C_{26}H_{24}O_6$ requires 432.1574); EI–MS *m*/*z* (rel. int): 432 $[M]^+$ (46), 417 $[M-Me]^+$ (100), 399 [417–H_2O]$^+$ (5), 277 (24), 213 (63), 216 $[M/2]^+$ (1), 208.5 [M−Me/2] (2), 167 [**a**]$^+$ (6), 149 [**a**–H_2O]$^+$ (4); *m** 402.5 (432–417), 236.8 (266–251), 184.0 (41–185).

Acknowledgements

We gratefully acknowledge the financial support of MIUR (Progetto di ricerca di Ateneo) and of the Agreement between CNR of Italy and Hungarian Academy of Sciences.

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