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ANTIMICROBIAL ISOFLAVANONES FROM *DESMODIUM CANUM*

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Key Word Index—*Desmodium canum*; Leguminosae; prenylated isoflavanones; antimicrobial activity.

Abstract—Bioassay-directed fractionation of *Desmodium canum* resulted in the isolation and characterization of three antimicrobial isoflavanones. These compounds, namely, desmodianones A, B and C, were assigned the structures 5,7,2′-trihydroxy-6,6″-dimethyl-6″-(4-methylpent-3-enyl)pyrano(2″,3″:4′,5′)isoflavanone, 5,2′,4′-trihydroxy-7-methoxy-6-methyl-8-(3-methylbut-2-enyl)-isoflavanone, and 5,7,2′,4′-tetrahydroxy-6-methyl-5′-(3,7-dimethylocta-2,6-dienyl)-isoflavanone, respectively.

INTRODUCTION

In a screening program [1] devised to detect antimicrobial activity in higher-plant extracts from the State of Pernambuco, Brazil, ethanolic extracts of roots of *Desmodium canum* (Gmell) Shintz and Tellung showed reproducible antimicrobial activity *in vitro* against *Bacillus subtilis* (9 IA-16), *Staphylococcus aureus* (IA-1), *Mycobacterium smegmatis* (IA-71) and *Streptococcus faecalis* (ATCC-6057). Three main compounds were isolated from the CH_2Cl_2-soluble portion of the ethanolic extract of the roots, where the majority of the activity was found to reside.

RESULTS AND DISCUSSION

The three compounds, desmodianones A (**1**), B (**2**), and C (**3**), according to the order of elution, showed the characteristic features of the isoflavanones [2], which are in general: (a) an ABC system of three doublets of doublets, attributable to H_2-2 and H-3, in the range 4.50–4.25 ppm of the ^{1}H NMR spectrum; (b) three signals in the ^{13}C NMR spectrum, at *ca* 71, 51 and 198 ppm, which can be assigned to the C-2, C-3 and C-4 carbons, respectively; (c) an absorbance at 1625 cm^{-1} in the IR spectrum; (d) absorption bands at 292 and 320 nm in the UV spectrum. For the desmodianones, the value of the C-3 resonance (47 ppm) was between that normally found for isoflavanones (51 ppm) and that typical of 2′ methoxyisoflavanones (45 ppm); the shift of this signal was thus attributed to the presence of a 2′-hydroxyl group in all three compounds. Another common feature of compounds **1**–**3** was the presence of an aromatic methyl group, which gave signals at δ2.0 in the ^{1}H NMR spectrum and δ7–8 in the ^{13}C NMR spectrum. The mass fragmentation (*vide infra*), the delayed bathochromic shift in the UV spectrum [3], and the resonance values in the ^{13}C NMR spectrum of the carbon bearing the above methyl group [4] are consistent with the aromatic methyl located at C-6.

The ^{1}H NMR spectrum of desmodianone A (**1**), $C_{26}H_{28}O_6$ by HRMS, showed signals for three hydroxy groups, one of which was chelated. Formation of the triacetate **1a** and the dimethyl ether **1b** provided further proof of the presence of hydroxyl groups. A bathochromic shift in the UV spectrum of **1** upon the addition of sodium acetate indicated the presence of a free hydroxyl group at position 7, while the singlet at *s* 6.07 in the ^{1}H NMR spectrum was attributed to the H-8 proton, thus revealing the substitution pattern of the A ring. This was confirmed by the presence of a peak at *m/z* 167 (*a* + H, $R = CH_3$ in Scheme 1; analysed for $C_8H_7O_4$) in the mass spectrum. In the B ring of **1**, where the third hydroxyl group must be located, two isolated aromatic protons (singlets at *s* 6.80 and 6.35 in the ^{1}H NMR spectrum) and a dihydropyran substituent (doublets at 6.45 and 5.49 with $J = 10$ Hz), which accounted for the sixth oxygen, were present. Only one aliphatic methyl resonance (at δ1.33) was present, whereas the remaining signals suggested an isohexenyl chain as the second substituent of the quaternary carbon of the chromene ring. The ^{1}H NMR data were in agreement with the values reported in the literature [5] for such a grouping. Because of the *para* relationship between the two aromatic protons in the B ring, the hydroxyl group must be located at the 2′-position, as anticipated by the value of the C-3

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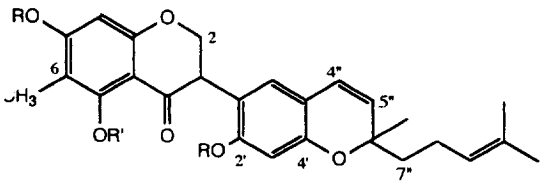
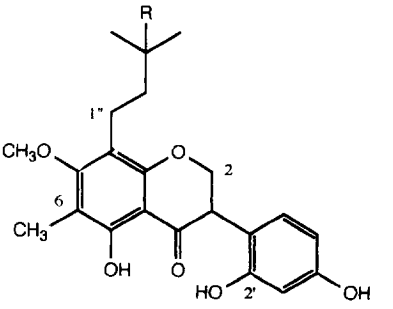
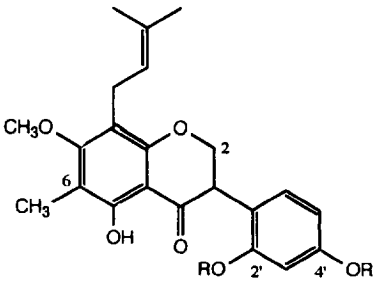


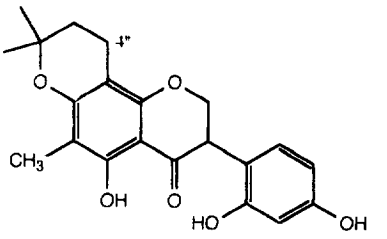
	R	R'
1	H	H
1a	$COCH_3$	$COCH_3$
1b	CH_3	H



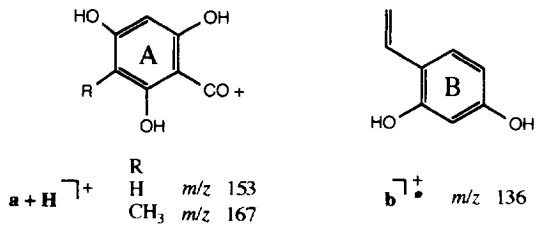
	R
2c	OCH_3
2d	OH
2e	Cl



	R
2	H
2a	$COCH_3$
2b	CH_3



2f



Scheme 1. Typical fragments of simple isoflavan [6].

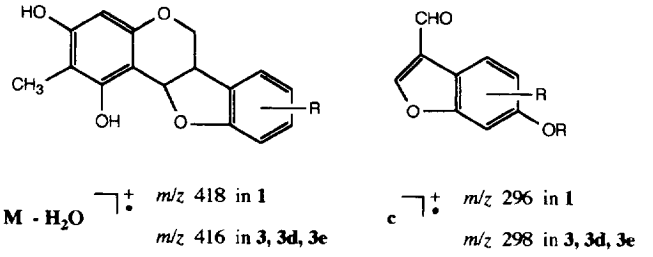
resonance. Moreover, on consideration of the resonances (δ155 and 157) of the two carbons involved, a 2',4'-oxygenation must be preferred to the alternative 2',5'-oxygenation, which could also be consistent with the proton substitution pattern. In conclusion, desmodianone A was assigned the structure 5,7,2'-trihydroxy-6,6''-dimethyl-6''-(4-methylpent-3-enyl) pyrano-(2'',3'':4',5')-isoflavanone (**1**). Under storage, desmodianone A gave a series of oxidation products (M^+ at *m/z* 452 and 468), in which a modification occurred in the isohexenyl chain (^{1}H NMR evidence); the structures of these products were not further investigated.

Desmodianone C (**3**), $C_{26}H_{30}O_6$ by HRMS, showed close spectral similarity with desmodianone A. Some coincident signals in the ^{1}H and ^{13}C NMR spectra, comparable UV spectra with additives, and the fragment at *m/z* 167 (**a** + H^+, R = CH_3 in Scheme 1) in the mass spectrum revealed an identical A ring. Two hydroxyl groups (shown by the ^{1}H NMR spectrum and by the formation of the derivatives **3a**, **3b** and **3c**) were now present in the ring B and were located in the 2',4'-positions, for the reasons discussed above. Conversely, the substituent at C-5' was identified as a geranyl chain by ^{1}H and ^{13}C NMR data [6].

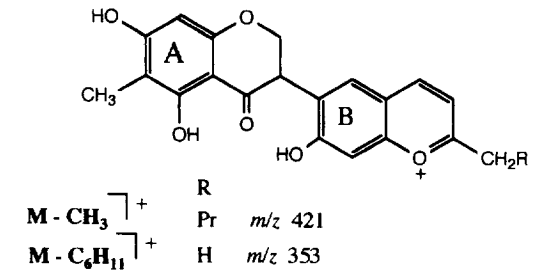
Desmodianone C was thus assigned the structure 5,7,2',4'-tetrahydroxy-6-methyl-5'-(3,7-dimethylocta-2,6-dienyl) isoflavanone (**3**). The nature of the 5'-substituent was confirmed by treatment with HCl in methanol of **3**, which gave the chromane derivatives **3d** and **3e**. Finally, as expected [5], the reaction of **3** with DDQ gave **1**, together with other unidentified products. The comparison of the mass spectra of compounds **1**, **3**, **3d** and **3e** strongly supports the assignment of the two structures and deserves the following comments. For the majority of flavonoids, the fragmentation of simple isoflavanones is regulated by a retro Diels–Alder reaction [7], which gives ions typical of the rings A (**a** + H^+, R = H, in Scheme (1) and **B** (**b** in Scheme 1). In 2'-hydroxyisoflavanones a further pathway is originated by the loss of H_2O [5], which very likely involves the enolic form of the carbonyl and the 2'-hydroxyl group (Scheme 2). Moreover, a McLafferty rearrangement of the ring C in the

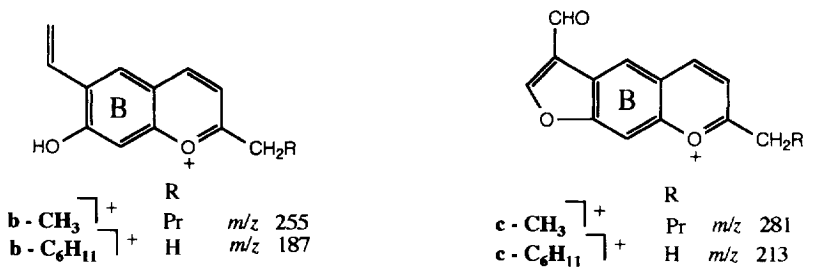
$[M - H_2O]^+$ ion, coupled with hydrogen transfer from C-2 to C-4, can be invoked to give the ion $[c]^+$, which is postulated in Scheme 2. In contrast, the fragmentation of complex isoflavanones appears, in general, to be dominated by the isoprenoid substituent, which is lost from the molecular ion to give either an oxonium or a tropylium ion. These ions yield fragments analogous to those originated by the molecular ion, but with a major intensity. The representative ions of these pathways of fragmentations are rationalized in Scheme 3 for desmodianone A and in Scheme 4 for desmodianone C. In the mass spectrum of **1** the expected loss of the C-6″ methyl group is flanked by that of the isohexenyl chain (C_6H_{11}) [5]. From both $[M - Me]^+$ and $[M - C_6H_{11}]^+$ ions originate two parallel fragmentations, leading to the *b*-type and the *c*-type ions. The loss of C_9H_{15} in desmodianone C, yields the analogous diagnostic fragments presented in Scheme 4. The structures assigned to the ions of Schemes 3 and 4 were supported by HRMS analysis (see Experimental).

Desmodianone B (**2**), $C_{22}H_{24}O_6$ by HRMS, displayed the signals of the following substituents in the ^1H NMR spectrum: three hydroxyl groups (one of which was chelated), an aromatic methyl group, a methoxyl group and an isopentenyl chain. The resonances of the three aromatic protons showed *ortho*, *ortho–meta* and *meta* couplings; the last two were shifted downfield in the ^1H NMR spectra of **2** in C_5D_5N [8] and of the acetyl-derivative **2a**. These results and the number of oxygenated carbons (four signals in the range 157–160 ppm of the ^{13}C NMR spectrum) are compatible with the presence of two hydroxyl groups in the positions 2′ and 4′ of the B ring. Conversely, the absence of a bathochromic shift after addition of sodium acetate in the UV spectrum and the value of the carbon resonance for a methoxyl group (60 ppm) required this substituent to be placed at C-7 position. The mass fragmentation of desmodianone B is rationalized in Scheme 5. The regular $[a + H]^+$ ion, shifted at *m/z* 249, undergoes the typical losses of the

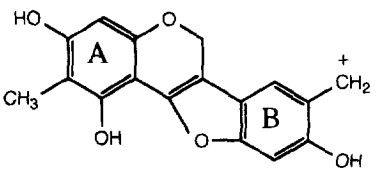


Scheme 2. Postulated fragments for 2′-hydroxyisoflavanones.

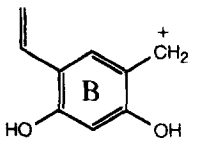




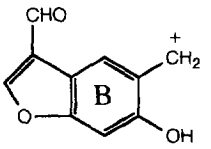
Scheme 3. Significant oxononium-type fragments in the mass spectrum of desmodianone A.



M - H_2O - C_9H_{15} $^{+}$ *m/z* 297

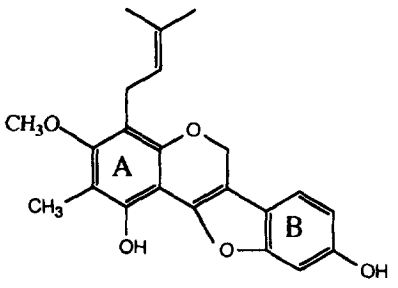


b - C_9H_{15} $^{+}$ *m/z* 149

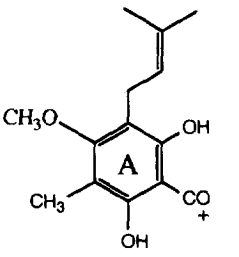


c - C_9H_{15} $^{+}$ *m/z* 175

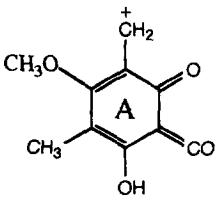
Scheme 4. Significant tropylium-like fragments in the mass spectrum of desmodianone C and its cyclicization products **3d** and **3e**.



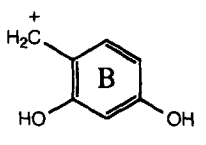
M - H_2O $^{+\bullet}$ *m/z* 366



a + H $^{+}$ *m/z* 249



a - C_4H_7 $^{+}$ *m/z* 193

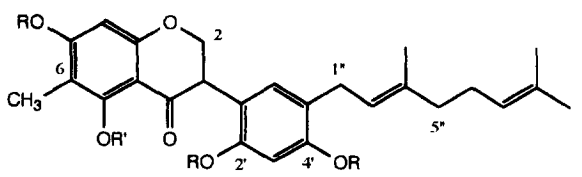


d $^{+}$ *m/z* 123

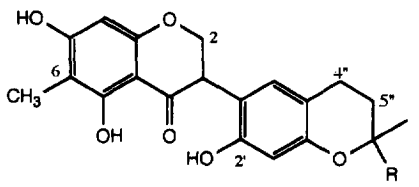
Scheme 5. Significant fragments in the mass spectrum of desmodianone B.

prenyl chain, mainly the loss of C_4H_7, which gives a tropylium ion. The unsubstituted ring B gives the ion *b* (see Scheme 1), as do lespedeol B [5] and simple isoflavanones [7] with an identical ring B. The fragment at *m/z* 123, analysed for $C_7H_7O_2$, can be assigned the tropylium structure *d*. An intense ion is present at the same *m/z* value in the mass spectrum of lespedeol B [5], but it may originate from the alkyl chain as C_9H_{15}.

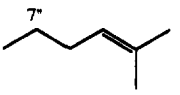
Although the presence of both the methyl and prenyl groups on ring A was confirmed by the mass fragmentation, the mutual position of the two alkyl substituents could not be assigned solely on the basis of the spectral data. Thus, the reactions of **2** with some Lewis acids were studied with the following results. Treatment of **2** with HCl in methanol gave the two addition products **2c** and **2d**, with no cyclicization occurring. Treatment of **2** with



	R	R'
3	H	H
3a	CH_3	H
3b	$COCH_3$	H
3c	$COCH_3$	$COCH_3$



R

3d 

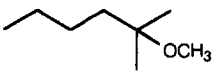
3e 

Table 1. *In-vitro* antimicrobial activity of isoflavanones **1** and **2** from *Desmodium canum*

Organism	Inhibitory concentration (μg/ml)	
	1	**2**
Bacillus subtilis (91A-16)	1–3	1–3
Staphylococcus aureus (IA-1)	5–10	3–5
Mycobacterium smegmatis (IA-71)	20–30	10–20
Streptococcus faecalis (ATCC-6057)	5–10	5–10
Escherichia coli S (IA-27)	i	50–100
Candida albicans (IA-1007)	50–100	50–100
Neurospora crassa (IA-2083)	i	50–100

i, No inhibition at 100 μg/ml.

BCl_3 gave compound **2e**, where HCl has been added to the double bond of the prenyl chain. Cumulatively, these experiments exclude the proximity of the prenyl chain and the chelated 5-hydroxyl. Finally, by treatment with BBr_3, **2** yielded a chromane derivative (**2f**), where the prenyl chain cyclized onto the free 7-hydroxy group, which only in under these drastic conditions had been formed by demethylation. In conclusion, desmodianone B was assigned the structure 5,2′,4′-trihydroxy-7-methoxy-6-methyl-8-(3-methyl-2-butenyl)-isoflavanone (**2**). Desmodianone B can be considered the 6,7-*O*-dimethyl derivative of kievitone, one of the major antifungal isoflavonoids produced by *Phaseolus vulgaris* in response to fungal infection [9].

The three desmodianones are optically active and showed intense positive Cotton effects in the 330–350 nm range in the CD curves. As 3*S*-isoflavanones exhibit the opposite behaviour in the ORD curves [10], a 3*R*-configuration can be assigned to desmodianones A, B and C. Pure isoflavanones **1** and **2** maintained the biological activity shown in the extracts. The antimicrobial potencies of compounds **1** and **2** in an *in vitro* dilution streak test are given in Table 1.

EXPERIMENTAL

General. All the isolated compounds and their derivatives were vitreous solids. Specroscopic measurements were conducted with the following instruments and media: IR, $CHCl_3$; UV, MeOH; EIMS, AEI MS 12, direct inlet; $[\alpha]_D$, Perkin Elmer 243, MeOH; CD, JASCO J-500A, MeOH; NMR (^1H and ^{13}C), Varian XL 300, Me_2CO-d_6 for **1**, **2**, **3**, **2e**, **2f**, **3d**, **3e** and $CDCl_3$ for **1a**, **1b**, **2a**, **2b**, **2c**, **2d**, **3a**, **3b** and **3c**. The ^{13}C NMR chemical shifts have been assigned by a comparison with the data reported for similar compounds [4, 6, 11]. The assignment of carbon chemical shifts of compound **2** was supported by an HETCOR experiment.

Plant material. Roots of *Desmodium canum* (Gmel.) Schinz and Tellung (Leguminosae) were collected in Paulista (Pe, Brazil) and identified by one of us (A.A.C.). A voucher specimen (IA-5168) has been deposited in the Herbarium of Departamento de Antibioticos, Recife, Pe, Brazil.

Extraction and fractionation. Ground air-dried roots (1.3 kg) were extracted with cold EtOH (×3) to give a residue (60 g), which was suspended in MeOH–H_2O, 93: 7 (400 ml). The insoluble material (3 g) was filtered off and the solution washed (×6) with hexane. The residue of the alcoholic solution was suspended in H_2O and extracted with CH_2Cl_2 (×3) and EtOAc (×3). Both the CH_2Cl_2 and the EtOAc extracts (17 g and 1 g, respectively), as also the residue (4 g) of the hexane washings showed antimicrobial activity. Conversely, the aq. solution was devoid of activity. Silica gel CC of part (7 g) of the CH_2Cl_2 extract with hexane–EtOAc, 7:3, gave a series of frs DD-I to DD-IX. Frs DD-II (1.2 g), DD-IV (0.8 g) and DD-VI (0.5 g) by extended chromatography on silica gel yielded desmodianone A (**1**, 0.85 g; with

$CHCl_3$–MeOH, 49:2), desmodianone B (**2**, 0.7 g; with hexane–EtOAc, 7:3) and desmodianone C (**3**, 0.35 g; with hexane–EtOAc, 3:1), respectively.

Desmodianone A (**1**). $[\alpha]_D^{22}$ + 32 (c = 0.9); UV λ_{max} nm (log ε): 292 (4.31), 319sh (3.91); (+ AcONa): 360; (+ $AlCl_3$): 320, 370 (after 20'); CD (c = 0.00012) $[\Phi]_{240}$ 0, $[\Phi]_{285}$ 2187, $[\Phi]_{330}$ 0, $[\Phi]_{350}$ + 1145; IR ν_{max} cm^{-1}: 3595, 3250, 1625, 1600, 1495, 1295, 1155, 1115, and 820; ^1H NMR: δ12.67 (1H, *s*, 5-OH), 9.50, 8.82 (1H each, *br s*, 7-OH, 2'-OH), 6.80 (1H, *s*, H-6'), 6.35 (1H, *s*, H-3'), 6.32 (1H, *d*, *J* = 10 Hz, H-4''), 6.03 (1H, *s*, H-8), 5.49 (1H, *d*, *J* = 10 Hz, H-5''), 5.11 (1H, *br t*, *J* = 7 Hz, H-9''), 4.57 (1H, *t*, *J* = 11 Hz, H-2ax), 4.44 (1H, *dd*, *J* = 11 and 5.5 Hz, H-2eq), 4.25 (1H, *dd*, *J* = 11 and 5.5 Hz, H-3), 2.1–1.9 (2H, *m*, H_2-8''), 1.98 (3H, *s*, 6-Me), 1.7–1.6 (2H, *m*, H_2-7''), 1.64, 1.57 (3H each, *br s*, 2 × Me), 1.29 (3H, *s*, 6''-Me); ^{13}C NMR: δ197.9 (*s*, C-4), 164.6 (*s*, C-7), 162.5, 161.7 (2 × *s*, C-5, C-8a), 156.7 (*s*, C-4'), 154.5 (*s*, C-2'), 131.6 (*s*, C-10''), 128.6 (*d*, C-6'), 126.9 (*d*, C-5''), 124.8 (*d*, C-9''), 122.9 (*d*, C-4''), 114.6, 114.3 (2 × *s*, C-1', C-5'), 104.5 (*s*, C-6), 103.9 (*d*, C-3'), 103.1 (*s*, C-4a), 94.7 (*d*, C-8), 79.0 (*s*, C-6''), 70.7 (*t*, C-2), 47.1 (*d*, C-3), 41.8 (*t*, C-7''), 26.7 (*q*, 6''-Me), 25.5 (*q*, *trans*-Me), 23.2 (*t*, C-6''), 17.4 (*q*, *cis*-Me), 6.9 (*q*, 6-Me); HREIMS *m/z* (rel. int.) $[M]^+$ 436.1906 (15) ($C_{26}H_{28}O_6$ requires 436.1886), $[M - Me]^+$ 421 (6), $[M - H_2O]^+$ 418 (3), $[M - C_6H_{11}]^+$ 353.1042 (92) ($C_{20}H_{15}O_5$ requires 353.1025), $[M - H_2O - C_6H_{15}]^+$ 335 (5), $[c]^+$ 296 (4), $[c - CH_3]^+$ 281 (4), $[b]^+$ 270 (5), $[b - CH_3]^+$ 255 (4), $[c - C_6H_{11}]^+$ 213.0563 (100) ($C_{13}H_9O_2$ requires 213.0552), $[b - C_6H_{11}]^+$ 187.0758 (26) ($C_{12}H_{11}O_2$ requires 187.0759), $[b - CO]^+$ 185.0595 (25) ($C_{12}H_9O_2$ requires 185.0603), $[a + H]^+$ 167.0345 (29) ($C_8H_7O_4$ requires 167.0344).

Triacetate (**1a**) (*with pyr/Ac_2O, overnight*). ^1H NMR: δ6.98 (1H, *s*, H-6'), 6.84 (1H, *s*, H-3'), 6.66 (1H, *s*, H-8), 2.36, 2.30 (6H, 3H, 3 × COMe).

Dimethyl ether (**1b**) (*with CH_2N_2*). ^1H NMR: δ12.42 (1H, *s*, 5-OH), 3.80, 3.76 (3H each, *s*, 2 × OMe).

Desmodianone B (**2**). $[\alpha]_D^{22}$ + 10.5 (*c* = 5.9); UV λ_{max} nm (log ε): 285 (4.14), 352sh (3.12); (+ AcONa): 285, 340sh; (+ $AlCl_3$): 285, 311, 361 (after 20'); CD (*c* = 0.000115), $[\Phi]_{240}$ 0, $[\Phi]_{285}$ − 2170, $[\Phi]_{296}$ 0, $[\Phi]_{312}$ + 870, $[\Phi]_{340}$ 0; IR ν_{max} cm^{-1}: 3590, 3300, 1630, 1600, 1500, 1165, 1152, 1130, 1115, 970, 830; ^1H NMR: δ 12.47 (1H, *s*, 5-OH), 8.55, 8.25 (1H each, *s*, 2'-OH, 4'-OH), 6.95 (1H, *d*, *J* = 8 Hz, H-6'), 6.45 (1H, *d*, *J* = 2.5 Hz, H-3'), 6.33 (1H, *dd*, *J* = 8 and 2.5 Hz, H-5'), 5.37 (1H, *br t*, *J* = 7 Hz, H-2''), 4.62 (1H, *t*, *J* = 11 Hz, H-2ax), 4.50 (1H, *dd*, *J* = 11 and 5.5 Hz, H-2eq), 4.27 (1H, *dd*, *J* = 11 and 5.5 Hz, H-3), 3.77 (3H, *s*, 7-OMe), 3.32 (2H, *d*, *J* = 7 Hz, H_2-1''), 2.07 (3H, *s*, 6-Me), 1.79, 1.71 (3H each, *br s*, 2 × Me); δ = δ(C_5D_5N) − δ(Me_2CO-d_6) = H-3' (+ 0.48), H-5' (+ 0.43), H-6' (+ 0.20); ^{13}C NMR: δ 200.2 (*s*, C-4), 165.5 (*s*, C-7), 160.6, 159.1, 158.9 (3 × *s*, C-5, C-8a, C-2'), 156.9 (*s*, C-4'), 131.9 (*d*, C-6'), 131.3 (*s*, C-3''), 124.2 (*d*, C-2''), 114.1, 113.8 (2 × *s*, C-8, C-1'), 111.3 (*s*, C-6), 107.7 (*d*, C-5'), 106.1 (*s*, C-4a), 103.7 (*d*, C-3'), 70.9 (*t*, C-2), 61.0 (*q*, OMe), 47.9 (*d*, C-3), 25.8 (*q*, *trans* Me), 22.9 (*t*, C-1''), 17.8 (*q*, *cis* Me), 8.3 (*q*, 6-Me); HREIMS *m/z* (rel. int.) $[M]^+$ 384.1570 (76) ($C_{22}H_{24}O_6$ requires 384.1572), $[M - H_2O]^+$ 366.1454 (54) ($C_{22}H_{22}O_5$ requires 366.1467), $[M - H_2O - Me]^+$ 351.1221 (35) ($C_{21}H_{19}O_5$ requires 251.1233), $[M - C_4H_7]^+$ 329.1025 (5) ($C_{18}H_{17}O_6$ requires 329.1025), $[M - H_2O - C_3H_7]^+$ 323.0910 (31) ($C_{19}H_{15}O_5$ requires 323.0920, $[M - C_5H_8]^+$ 316.0930 (8) ($C_{17}H_{16}O_6$ requires 316.0947), $[M - H_2O - C_4H_7]^+$ 311.0905 (13) ($C_{18}H_{15}O_5$ requires 311.0911), $[M - H_2O - C_4H_8]^+$ 310.0843 (12) ($C_{18}H_{14}O_5$ requires 310.0841), $[M - H_2O - C_5H_8]^+$ 298.0828 (12) ($C_{17}H_{14}O_5$ requires 298.0841), $[a + H]^+$ 249.1129 (39) ($C_{14}H_{17}O_4$ requires 249.1127), $[a - H]^+$ 247.0977 (42) ($C_{14}H_{15}O_4$ requires 247.0971), $[a - C_4H_7]^+$ 192.9920 ($C_{10}H_9O_4$ requires 193.0501), $[a - C_5H_7]^+$ 181.0498 ($C_9H_9O_4$ requires 181.0501), $[b]^+$ 136.0520 (28) ($C_8H_8O_2$ requires 136.0524), $[d]^+$ 123.0447 (36) ($C_7H_7O_2$ requires 123.0445); m* 348.8 (384 → 366), 285.0 (366 → 323), 218.9 (248 → 233), 191.0 (220 → 205), 176.1 (205 → 190), 149.6 (249 → 193), 137.1 (193 → 163).

Diacetate (**2a**) (*with pyr/Ac_2O, after 3 h*). ^1H NMR: δ12.0 (1H, *s*, 5-OH), 7.24 (1H, *d*, *J* = 8 Hz, H-6'; + 0.27), 7.06 (1H, *d*, *J* = 2.5 Hz, H-3'; + 0.59), 7.01 (1H, *dd*, *J* = 8 and 2.5 Hz, H-5'; + 0.66), 2.27, 2.24 (3H each, *s*, 2 × COMe).

Dimethyl ether (**2b**) (*with CH_2N_2*). ^1H NMR: δ12.30 (1H, *s*, 5-OH), 3.78, 3.76 (6H, 3H, *s*, 3 × OMe); EIMS *m/z* (rel. int.) $[M]^+$ 412 (56) $[M - Me]^+$ 397 (3), $[M - C_4H_7]^+$ 357 (1), [a]$^+$ 248 (5), 247 (1), $[a - Me]^+$ 233 (37), $[a - CO]^+$ 220 (27), $[a - COMe]^+$ 205 (20), $[a - C_4H_7]^+$ 193 (12), $[b]^+$ 164 (52), $[d]^+$ 151 (100), $[b - Me]^+$ 149 (16); m* 218.9 (248 → 233), 191.0 (220 → 205), 135.4 (164 → 149).

Reactions of 2 with Lewis Acids. (a) With HCl: **2** (80 mg) in MeOH (16 ml) and HCl conc (4 ml) was held at reflux for 2 hr. The reaction mixture was concd, H_2O added and mixture extracted with $CHCl_3$ (× 3). The residue of the pooled extracts on silica gel CC with hexane–EtOAc, 2:1, yielded compounds **2c** (40 mg) and **2d** (25 mg). Compound **2c**: ^1H NMR δ12.46 (1H, *s*, 5-OH), 8.66, 8.35 (1H each, *br s*, 2-OH', 4-OH'), 6.97 (1H, *d*, *J* = 8.5 Hz, H-6'), 6.45 (1H, *d*, *J* = 2.5 Hz, H-3'), 6.34 (1H, *dd*, *J* = 8.5 and 2.5 Hz, H-5'), 4.66 (1H, *t*, *J* = 11 Hz, H-2ax), 4.52 (1H, *dd*, *J* = 11 and 5.5 Hz, H-2eq), 4.31 (1H, *dd*, *J* = 11 and 5.5 Hz, H-3), 3.81 (3H, *s*, 7-OMe), 3.20 (3H, *s*, OMe), 2.58 (2H, *br t*, H_2-α), 2.06 (3H, *s*, 6-Me), 1.62 (2H, *m*, H_2-β), 1.18 (6H, *br s*, 2 × Me); EIMS *m/z* (rel. int.): $[M]^+$ 416 (47), $[M - H_2O]^+$ 398 (6), $[M - MeOH]^+$ 384 (22), 369 (6), $[M - H_2O - MeOH]^+$ 366 (19), $[366 - Me]^+$ (8), $[384 - C_4H_7]$ 329 (68), 328 (100), 323 (9), $[366 - C_4H_7]^+$ 311 (34), 310 (21), $[a + H]^+$ 249 (15), 248 (7), 247 (16), 233 (24), 220 (20), 205 (18), $[a - C_4H_7]^+$ 193 (50), $[b]^+$ 136 (32), 135 (29), $[d]^+$ 123 (44); m* 354.5 (416 → 384), 348.8 (384 → 366), 294.0 (329 → 311), 258.6 (416 → 328). Compound **2d**: ^1H NMR: δ12.45 (1H, *s*, 5-OH), 8.64, 8.33 (1H each, *br s*, 2'-OH, 4'-OH), 6.97 (1H, *d*, *J* = 8.5 Hz, H-6'), 6.45 (1H, *d*, *J* = 2.5 Hz, H-3'), 6.34 (1H, *dd*, *J* = 8.5 and 2.5 Hz, H-5'), 4.65 (1H, *t*, *J* = 11 Hz, H-2ax), 4.51 (1H, *dd*, *J* = 11 and 5.5 Hz, H-2eq), 4.30 (1H, *dd*, *J* = 11 and 5.5 Hz, H-3), 3.81) (3H, *s*, 7-OMe), 2.67 (2H, *t*, *J* = 8.5 Hz, H_2 - α), 2.05 (3H, *s*, 6-Me), 1.64 (2H, *m*,

H_2-β), 1.24 (6H, *br s*, 2 × Me); EIMS *m/z* (rel. int.): $[M]^+$ 402 (27), $[M - H_2O]^+$ 384 (16), $[M - 2H_2O]^+$ (8), [366 – Me] 351 (4), $[384 - C_4H_7]^+$ 329 (39), 328 (64), $[366 - C_4H_7]^+$ 311 (27), $[a + H]^+$ 249 (21), 248 (12), 247 (9), $[a - C_4H_7]^+$ 193 (100), $[b]^+$ 136 (55), 135 (54), $[d]^+$ 123 (80). (b) With BCl_3: BCl_3 1 M in CH_2Cl_2 (1.5 ml) was added to a cooled solution of **2** (100 mg) in CH_2Cl_2 (8.5 ml) and the mixture was left standing at 0° overnight. After evapn the reaction mixture was dissolved in MeOH and left overnight. The residue on silica gel CC with CH_2Cl_2–hexane–EtOAc, 2:2:1, yielded **2e** (90 mg). Compound **2e**: ^{1}H NMR: δ12.46 (1H, *s*, 5-OH), 8.65, 8.32 (1H each, *br s*, 2′-OH, 4′-OH), 6.97 (1H, *d*, J = 8.5 Hz, H-6′), 6.46 (1H, *d*, J = 2.5 Hz, H-3′), 6.35 (1H, *dd*, J = 8.5 and 2.5 Hz, H-5′), 4.65 (1H, *t*, J = 11 Hz, H-2ax), 4.51 (1H, *dd*, J = 11 and 5.5 Hz, H-2eq), 4.30 (1H, *dd*, J = 11 and 5.5 Hz, H-3), 2.66 (2H, *t*, J = 8.5 Hz, H-2α) 2.05 (3H, *s*, 6-Me), 1.66 (2H, *m*, H_2-β), 1.25 (6H, *s*, 2 × Me); EIMS *m/z* (rel. int.) $[M + 2]^+$ 422 (6), $[M]^+$ 420 (18), 422 (6), 404 (2), $[M - H_2O]^+$ 402 (6), $[M - HCl]^+$ 384 (100), $[384 - H_2O]$ 366 (97), $[M - C_4H_8Cl]^+$ 311 (45), $[a + H]^+$ 249 (18), 247 (16), $[a - C_4H_7]^+$ 193 (44), $[b]^+$ 136 (12), 135 (7), $[d]^+$ 123 (23). (c) With BBr_3: BBr_3 1 M in CH_2Cl_2 (0.7 ml) was added to compound **2** (50 mg) in CH_2Cl_2 (4.3 ml) at 0°C. The reaction mixture was left standing at room temp. overnight. The residue obtained by standard work up gave, on silica gel CC with CH_2Cl_2–hexane, 4:1, **2f** (25 mg). Compound **2f**: ^{1}H NMR: δ12.41 (1H, *s*, OH-5), 8.66, 8.35 (1H each, 2′-OH, 4′-OH), 6.93 (1H, *d*, J = 8.5 Hz, H-6′), 6.46 (1H, *d*, J = 2.5 Hz, H-3′), 6.30 (1H, *dd*, J = 8.5 + 2.5 Hz, H-5′), 4.63 (1H, *t*, J = 11 Hz, H-2ax), 4.51 (1H, *dd*, J = 11 + 5.5 Hz, H-2eq), 4.24 (1H, *dd*, J = 11 + 5.5 Hz, H-3), 2.60 (2H, *br t*, J = 7.5 Hz, H_2-α), 1.93 (3H, *s*, 6-Me), 1.81 (2H, *br t*, J = 7.5 Hz, H_2-β), 1.36, 1.35 (3H each, *s*, 2 × Me); EIMS *m/z* (rel. int.) $[M]^+$ 370 (100), $[M - H_2O]^+$ 352 (29), 315 (6), $[M - C_4H_8]^+$ 314 (6), 297 (18), $[352 - C_4H_8]^+$ (18), $[a + H]^+$ 235 (92), 234 (96), $[a - C_4H_7]^+$ 179 (86), $[b]^+$ 136 (19), 135 (9), $[d]^+$ 123 (19).

Desmodianone C (**3**). $[\alpha]_D^{22}$ + 9 (c = 0.6); UV λ_{max} (log ε) nm: 293 (4.12), 330sh (3.22); (+ AcONa): 290, 338; (+ $AlCl_3$): 310, 360 (after 20′); CD (C = 0.0001), $[\Phi]_{240}$ 0, $[\Phi]_{288}$ – 1300, $[\Phi]_{338}$ 0, $[\Phi]_{348}$ + 630; IR ν_{max} cm^{-1}: 3590, 3260, 1630, 1600, 1495, 1150, 1108, 820; ^{1}H NMR: δ 12.67 (1H, *s*, 5-OH), 9.57, 8.36, 8.16 (1H each, *s*, 7-OH, 2′-OH, 4′-OH), 6.82 (1H, *br s*, H-6′), 6.47 (1H, *s*, H-3′), 6.02 (1H, *s*, H-8), 5.29 (1H, *t*, J = 7.5 Hz, H-2″), 5.11 (1H, *m*, H-6″), 4.57 (1H, *t*, J = 11 Hz, H-2ax), 4.42 (1H, *dd*, J = 11 and 5.5 Hz, H-2eq), 4.18 (1H, *dd*, J = 11 and 5.5 Hz, H-3), 3.20 (2H, *d*, J = 7.5 Hz, H-1″), 2.05–1.95 (4H, *m*, H_2-4″, H_2-5″), 1.97 (3H, *s*, 6-Me), 1.64 (6H, *br s*, 2 × Me), 1.58 (3H, *s*, Me); ^{13}C NMR: δ198.2 (*s*, C-4), 164.4 (*s*, C-7), 162.4, 161.6 (2 × *s*, C-5, C-8a), 155.5, 154.3 (2 × *s*, C-4′, C-2′), 135.8 (*s*, C-3″), 131.3 (*s*, C-8″), 131.1 (*d*, C-6′), 124.8, 123.8 (2 × *d*, C-2″, C-7″), 119.6 (*s*, C-1′), 113.4 (*s*, C-5′), 104.4 (*s*, C-6), 103.4 (*d*, C-3′), 103.3 (*s*, C-4a), 94.6 (*d*, C-8), 70.7 (*t*, C-2), 46.9 (*d*, C-3), 40.0 (*t*, C-5″), 27.8, 27.1 (2 × *t*, C-1″, C-6″), 25.5 (*q*, *trans*-Me), 17.5 (*q*, *cis*-Me), 6.8 (*q*, 6-Me); HREIMS *m/z* (rel. int.) $[M]^+$ 438.2056 (21) ($C_{26}H_{30}O_6$ requires 438.2043), $[M - H_2O]^+$ 420 (4), $[M - C_6H_{13}]^+$ 353.1051 (19) ($C_{20}H_{17}O_6$ requires 353.1025), $[M - C_9H_{15}]^+$ 315.0877 (69) ($C_{17}H_{15}O_6$ requries 315.0869), 314 (8), $[c]^+$ 298 (5), $[M - H_2O - C_9H_{15}]^+$ 297.0777 (10) ($C_{17}H_{13}O_5$ requires 297.0763), $[b]^+$ 272.1778 (21) ($C_{18}H_{24}O_2$ requires 272.1777), $[c - CO]^+$ 270 (6), $[c - C_9H_{15}]^+$ 175.0397 (59) ($C_{10}H_7O_3$ requires 175.0396), 174 (4), $[a + H]^+$ 167.0345 (100) ($C_8H_7O_4$ requires 167.0345), $[b - C_9H_{15}]^+$ 149.0609 (56) ($C_9H_9O_2$ requires 149.0602), $[c - CO - C_9H_{15}]^+$ 147.0440 (15) ($C_9H_7O_2$ requires 147.0446), $[C_9H_{15}]^+$ 123 (13).

Trimethyl derivative (**3a**) (*with* CH_2N_2). ^{1}H NMR: δ12.40 (1H, *s*, 5-OH), 3.80, 3.76 (6H, 3H, *s*, 3 × OMe).

Triacetyl derivative (**3b**) (*with pyr/*Ac_2O, *after 3 h*). ^{1}H NMR: δ12.15 (1H, *s*, OH-5), 7.05 (1H, *s*, H-3′), 6.99 (1H, *br s*, H-6′), 6.27 (1H, *s*, H-8), 2.36, 2.30 (3H, 6H, *s*, 3 × COMe).

Tetracetyl derivative (**3c**) (*with pyr/*Ac_2O *overnight*). ^{1}H NMR: δ7.05 (1H, *s*, H-3′), 6.99 (1H, *br s*, H-6′), 6.67 (1H, *s*, H-8), 2.36, 2.30 (6H, 6H, *s*, 4 × COMe).

Cyclicization of **3**. Desmodianone C (**3**, 100 mg) in MeOH (20 ml and conc HCl (5 ml) was held at reflux for 2 hr. The reaction mixture was concd, H_2O added and mixture extracted with $CHCl_3$ (× 3). The residue of the organic fractions on silica gel CC with hexane–EtOAc, 3:1, yielded compounds **3d** (22 mg) and **3e** (56 mg).

Cyclodesmodianone C-1 (**3d**). ^{1}H NMR: δ12.67 (1H, *s*, 5-OH), 9.62, 8.52 (1H each, *s*, 7-OH, 2′-OH), 6.81 (1H, *br s*, H-6′), 6.31 (1H, *s*, H-3′), 6.04 (1H, *s*, H-8), 5.13 (1H, *br t*, J = 6.5 Hz, H-9″), 4.59 (1H, *t*, J = 11 Hz, H-2ax), 4.43 (1H, *dd*, J = 11 and 5.5 Hz, H-2eq), 4.22 (1H, *dd*, J = 11 and 5.5 Hz, H-3), 2.64 (2H, *br t*, J = 6.5 Hz, H_2-4″), 2.0–1.95 (2H, *m*, H_2-8″), 1.98 (3H, *s*, 6-Me), 1.81, 1.72 (1H each, *dd*, J = 14 and 6.5 Hz, H_2-5″), 1.6–1.5 (2H, *m*, H_2-7″, 1.65, 1.60 (3H each, *s*, 10″-Me_2), 1.29 (3H, *s*, 6″-Me); HREIMS *m/z* (rel. int.): $[M]^+$ 438.2065 (39) ($C_{26}H_{30}O_6$ requires 438.2043), $[M - H_2O]^+$ 420.1941 (32) ($C_{26}H_{28}O_5$ requires 420.1937), $[M - C_9H_{15}]^+$ 315.0869 (85) ($C_{17}H_{15}O_6$ requires 315.0869), $[c]^+$ 298 (16), $[M - C_9H_{15} - H_2O]^+$ 297.0768 (41) ($C_{17}H_{13}O_5$ requires 297.0763), 296 (40), $[b]^+$ 272.1779 (18), ($C_{18}H_{24}O_2$ requires 272.1777), $[c - CO]^+$ 270 (2), $[c - C_9H_{15}]^+$ 175.0390 (95) ($C_{10}H_7O_3$ requires 175.0395), $[a + H]^+$ 167.0336 (88) ($C_8H_7O_4$ requires 167.0345), $[b - C_9H_{15}]^+$ 149.0610 (100) ($C_9H_9O_2$ requires 149.0602), $[c - CO - C_9H_{15}]^+$ 147.0453 (42) ($C_9H_7O_2$ requires 147.0446), $[C_9H_{15}]^+$ 123 (30).

Cyclodesmodianone C-2 (**3e**). ^{1}H NMR: δ12.67 (1H, *s*, OH-5), 9.59, 8.52 (1H each, *s*, OH-7, OH-2′), 6.81 (1H, *br s*, H-6′), 6.31 (1H, *s*, H-3′), 6.03 (1H, *s*, H-8), 4.59 (1H, *t*, J = 11 Hz, H-2ax), 4.43 (1H, *dd*, J = 11 and 5.5 Hz, H-2eq), 4.22 (1H, *dd*, J = 11 and 5.5 Hz, H-3), 3.11 (3H, *s*, 10″-OMe), 2.63 (2H, *t*, J = 6.5 Hz, H_2-4″), 1.98 (3H, *s*, 6-Me), 1.80, 1.72 (1H each, *dd*, J = 13.5 and 6.5 Hz, H_2-5″), 1.57 (2H, *m*, H_2-8″, 1.44 (4H, *m*, H_2-7″, H_2-9″), 1.25 (3H, *s*, 6″-Me), 1.11 (6H, *s*, 2 × Me); HREIMS *m/z* (rel. int.): $[M]^+$ 470.2314 (21) ($C_{27}H_{34}O_7$ requires 470.2305), $[M - Me]^+$ 455 (4), $[M - H_2O]^+$ 452 (5), $[M - MeOH]^+$ 438.2025 (37), ($C_{26}H_{30}O_6$ requires

438.2042), $[M - MeOH - H_2O]^+$ 420.1917 (6) ($C_{26}H_{28}O_5$ requires 420.1936), $[M - MeOH - C_9H_{15}]^+$ 315.0861 (100) ($C_{17}H_{15}O_6$ requires 315.0868), 314 (30), $[c]^+$ 298 (4), $[M - H_2O - C_9H_{15}]^+$ 297 (7), 296 (7), $[b]^+$ 272.1779 (18) ($C_{14}H_{14}O_2$ requires 272.1777), $[c - CO]^+$ 270 (2), $[c - C_9H_{15}]^+$ 175.0390 (65) ($C_{10}H_7O_3$ requires 175.0395), $[a + H]^+$ 167.0335 (56) ($C_8H_7O_4$ requires 167.0345), $[b - C_9H_{15}]^+$ 149.0601 (64) ($C_9H_9O_2$ requires 149.0603), $[c - CO - C_9H_{15}]^+$ 147 (17), $[C_9H_{15}]^+$ 123 (7), $[Me_2C = OMe]$ 73 (71).

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