Constituents of 'Dragon's Blood.' Part 4.[1] Dracoflavan A, a Novel Secotriflavanoid

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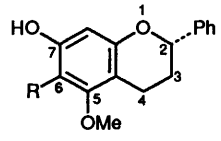
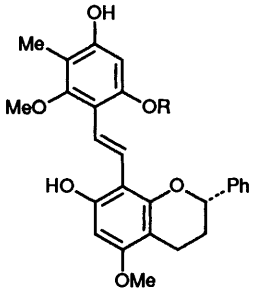
A novel secotriflavonoid with two flavan nuclei was isolated from the resin 'Dragon's blood.' Its structure and absolute configuration were established by chemical and spectroscopic means.

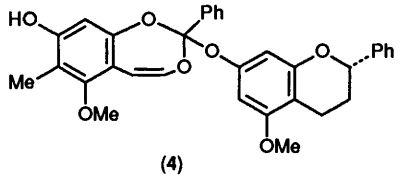
'Dragon's blood' is a commercially available resin which is obtained, in the form of granules or a reddish powder, from the exudates of the fruits of *Daemonorops draco* (Palmae), a palm growing in Southeast Asia.[2] Previous research on the flavonoid constituents of the resin concerned red pigments,[3] flavans such as compounds (**1**) and (**2**),[4] a secobiflavanoid (**3**),[5] and, recently, draco-oxepine (**4**),[1] a new biflavanoid with an unusual benzodioxepine moiety.



(**1**) R = Me
(**2**) R = H



(**3**) R = Bz
(**3a**) R = H

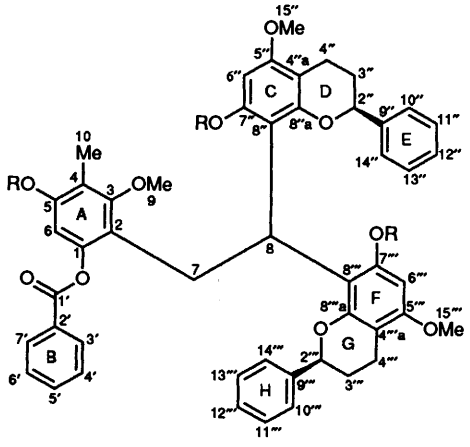


(**4**)

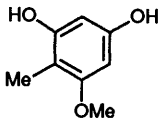
Further investigations on the resin resulted in the isolation of a new trimeric flavanoid compound, which we named dracoflavan A (**5**). In this paper we describe the complete structural elucidation of compound (**5**) on the basis of chemical evidence and NMR experiments.

Dracoflavan A (**5**) was obtained by extraction of the resin with ethyl acetate, followed by chromatography, as an optically active white powder. It showed an UV spectrum consistent with a flavanoid structure (λ_{max} 214, 272, and 280sh nm; ε 38 500, 2 500, and 2 300),[6] while IR and NMR data were indicative of both the presence of an ester group (ν 1 730 cm^{-1}; δ_C 164.85) and three phenolic hydroxy protons (ν 3 350 cm^{-1}; δ_H 8.28, 8.13, and 8.03). The formation of the triacetate (**5a**) upon acetylation confirmed the presence of the hydroxy protons.

Treatment of compound (**5**) with HCl in methanol gave a mixture containing (2*S*)-5-methoxyflavan-7-ol (**2**) and 1-*O*-methyl-2-methylphloroglucinol (**6**), indicating that dracoflavan



(**5**) R = H
(**5a**) R = Ac



(**6**)

A (**5**) is structurally related to the main constituents of the resin, *viz.* the flavans (**1**) and (**2**).

FAB mass spectrometry indicated the molecular formula $C_{49}H_{46}O_{10}$, (MH^+ 795), thus suggesting that compound (**5**) possesses a triflavonoid structure. A prominent peak at *m/z* 538 ($M^+ - 256$) indicated the loss of a monosubstituted methoxyflavanol unit such as that of structure (**2**), while the loss of 104 mass units from the peak at *m/z* 538 could be rationalized as being due to the extrusion of styrene from a second unit such as compound (**2**) (retro-Diels–Alder from a flavan moiety).[6]

The ^1H and ^{13}C NMR spectra of dracoflavan A (**5**) (Tables 1 and 2) confirmed the presence of two monosubstituted methoxy flavanoid moieties. The assignment of the signals belonging to these two moieties was straightforward, by comparison with similar structures[1,5] and on chemical-shift criteria. Moreover, the nuclear Overhauser effects (NOEs) observed between 6″-H and the 7″-hydroxy and the 15″-*O*-methyl protons (5 and 20%, respectively) and those similarly observed between the corresponding protons of ring F, and the fact that rings C and F both contain only one aromatic proton, indicated that these two rings are substituted at C-8″ and C-8‴.

Table 1. ^1H NMR chemical shifts for dracoflavan A (**5**) in [$^2H_6$]acetone.

Proton[a]	δ_H[b]
6	6.42
7	3.37
8	5.32
9	3.33
10	1.92
3′	7.84
4′	7.43
5′	7.62
6′	7.43
7′	7.84
5-OH	8.28
2″, 2‴	4.79, 4.75
3″, 3‴	1.95, 1.95
4″, 4‴	2.52, 2.52
6″, 6‴	5.94, 5.89
15″, 15‴	3.66, 3.65
7″-OH, 7‴-OH	8.13, 8.03

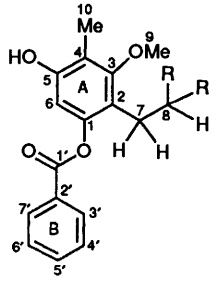
[a] The protons of rings E and H resonate between δ_H 7.1–7.4. [b] The corresponding proton resonances of the two flavanyl moieties can be interchanged.

Table 2. ^{13}C NMR chemical shifts for dracoflavan A (**5**) in [$^2H_6$]acetone.

Carbon	δ_C	Carbon	δ_C[a]
1	148.66	2″, 2‴	78.67, 78.60
2	120.27	3″, 3‴	29.77, 29.19
3	159.65	4″, 4‴	20.09, 19.74
4	115.86	4″a, 4‴a	102.99, 102.84
5	154.86	5″, 5‴	157.26, 157.21
6	105.68	6″, 6‴	93.35, 93.41
7	29.19	7″, 7‴	155.31, 155.69
8	32.60	8″, 8‴	110.99, 110.50
9	60.78	8″a, 8‴a	153.99, 153.85
10	9.70	9″, 9‴	142.63, 142.48
1′	164.85	10″, 10‴	126.99, 126.90
2′	130.80	11″, 11‴	129.10, 129.00
3′	130.87	12″, 12‴	128.26, 128.21
4′	129.04	13″, 13‴	129.10, 129.00
5′	133.82	14″, 14‴	126.99, 126.90
6′	129.04	15″, 15‴	55.45, 55.45
7′	130.87		

[a] The corresponding carbon resonances of the two flavanyl moieties can be interchanged.

The analysis of the ^1H and ^{13}C NMR signals contained in the remaining $C_{17}H_{16}O_4$ portion of the molecule, together with the use of a ^1H, ^{13}C heteronuclear correlation (HETCOR) spectrum to identify the specific resonances associated with each protonated carbon, permitted us to construct the partial structure (**A**).



(**A**)

Table 3. Selected ^{13}C–^1H coupling constants for the part structure (**A**) in [$^2H_6$]acetone.

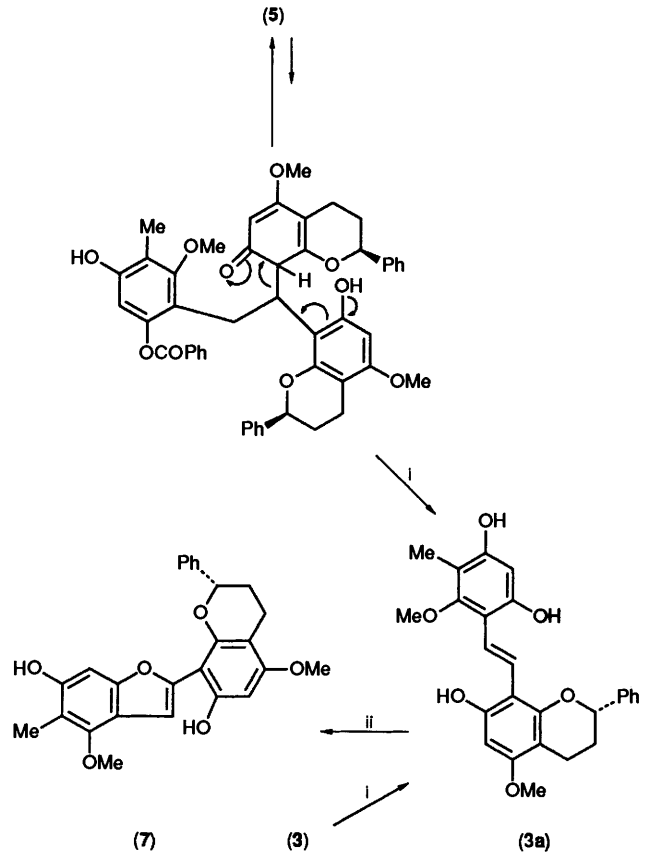
J(CH)	Hz	J(CH)	Hz
C-1, 6-H	4.5	C-6, 6-H	161
C-1, 7-H_2	4.5	C-6, 5-OH	4
C-2, 6-H	5	C-7, 7-H_2	132
C-2, 7-H_2	5	C-7, 8-H	7.5
C-2, 8-H	4.5	C-8, 7-H_2	5
C-3, 7-H_2	4	C-8, 8-H	129
C-3, 9-H_3	4	C-9, 9-H_3	143.5
C-3, 10-H_3	4	C-10, 10-H_3	127.5
C-4, 6-H	5	C-1′, 3′-H	4
C-4, 10-H_3	6	C-1′, 7′-H	4
C-4, 5-OH	6	C-2′, 4′-H	7.5
C-5, 6-H	3.5	C-2′, 6′-H	7.5
C-5, 10-H_3	4	C-3′, 3′-H	163
C-5, 5-OH	3.5	C-4′, 4′-H	161
		C-5′, 5′-H	161.5

Specifically, the ^1H NMR spectrum exhibited an AA′BB′C spin system attributable to the five aromatic protons of ring B, one aromatic proton and one phenolic hydroxy proton (6-H and 5-OH), one OMe and one Me group (9- and 10-H_3) and a C(7)H_2–C(8)H grouping ($^3J_{7,8}$ 6.5 Hz). Accordingly, the ^{13}C NMR spectrum showed signals (Table 2, column 1) which were assigned to the six methine and six quaternary sp^2 carbon atoms of rings A and B, and four resonances between δ_C 60.78–9.70 attributable to two methyl (C-9 and C-10), one methylene (C-7), and one methine (C-8) sp^3 carbon atoms. Furthermore, it revealed the presence of a benzoate carbonyl carbon atom at δ_C 164.85, which was assigned to C-1′ as it presented three-bond (C, H) couplings of 4 Hz with 3′-H and 7′-H. Extensive use of the ^{13}C–{^1H} low-power selective decouplings, the results of which are reported in Table 3, allowed the determination of the substitution pattern of ring A. Selective irradiation of the 9-*O*-methyl protons affected the oxygen-bearing carbon atom three bonds removed, which was therefore assigned to C-3 [^3J(C,H) 4 Hz]. This carbon, in turn, presented (C, H) couplings of 4 Hz with 7-H_2 and 10-H_3 which are through necessity over three bonds, this fact indicating that the C-7 methylene and the C-10 methyl groups must be located at C-2 and C-4, respectively. The OH group was allocated at C-5 since C-4 and C-5 were both coupled to the 5-hydroxy and 10-methyl protons [^2J(C-4, 10-H_3) 6, ^3J(C-4, 5-OH) 6, ^2J(C-5, 5-OH) 3.5, and ^3J(C-5, 10-H_3) 4 Hz], while the unsubstituted carbon, which presented a three-bond coupling of 4 Hz with the 5-hydroxy proton, was assigned to C-6. Finally, the benzoate moiety was placed at C-1 since this carbon, resonating at δ_C 148.66, was coupled *via* a three-bond interaction of 4.5 Hz to 7-H_2. The NOEs observed between 5-OH and 10-H_3 (1%), between 9-H_3 and 10-H_3 (3%), and between 5-OH and 6-H (19%) were in accord with the above evidence.

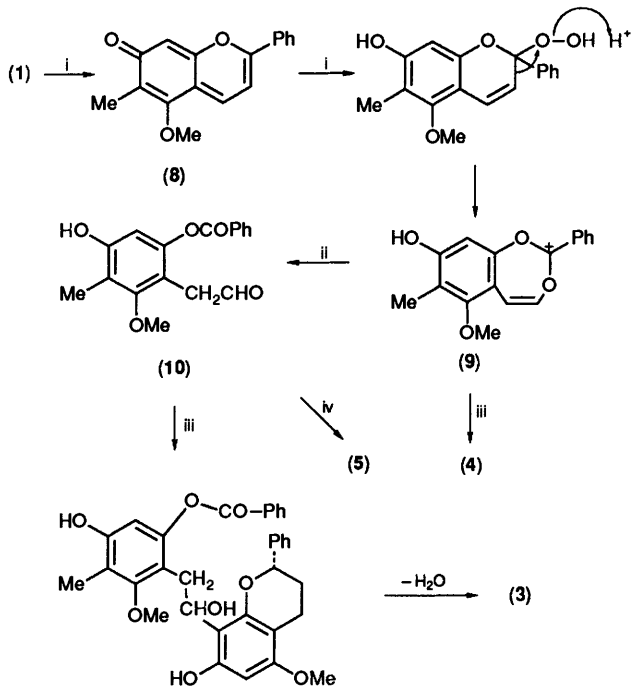
In order to obtain the complete structure of dracoflavan A (**5**), we had only to link C-8 to C-8″ and C-8‴. The presence of three-bond (C, H) couplings of 5.5 Hz between 8-H and the carbons at δ_C 155.31 and 155.69, assigned to C-7″ and C-7‴ as they were also coupled to 6″-H and 7″-OH and to 6‴-H and 7‴-OH [J(CH) 3–4 Hz], gave further support to this conclusion.

The absolute configuration of compound (**5**) at carbons 2″ and 2‴ is *S*, as shown by the isolation of 2*S*-(**2**) from the acidic degradation reaction products. As the two flavan moieties have the same configuration, the central carbon atom C-8 is not asymmetric.

The behaviour of dracoflavan A (**5**) upon alkaline degradation with methanolic KOH is also consistent with the



Scheme 1. *Reagents:* i, OH^-; ii, OX.



Scheme 2. *Reagents:* i, OX; ii, water; iii, (2); iv, 2 × (2).

proposed structure (Scheme 1). In fact, we obtained as the major product the benzofuran (7), a compound that we had formerly[5] isolated from a similar alkaline treatment of the secobiflavanoid (3). The formation of compound (7) may be explained as occurring through aerial oxidation of the phenol (3a) which, in turn, can arise from compound (5) by hydrolysis of the benzoate ester group and elimination of the flavan (2), and from compound (3) by the hydrolysis of the benzoate.

We have already commented[7] on the unusual structures of the flavonoid constituents of 'Dragon's blood,' whose formation in the resin can be explained with different oxidation processes, all starting from the monomeric flavans (1) and (2), and most probably due to aerial oxidation in the solid state. In the particular case of dracoflavan A (5), a possible pathway to account for its formation from the precursors (1) and (2) may be rationalized as a preferential oxidation of compound (1) (*cf.* ref. 4) very similar to that postulated for the formation of draco-oxepine (4)[1] (Scheme 2). Thus the benzodioxepinium ion (9), obtained by a Baeyer–Villiger-type oxidation[8] of dracorhodin (8),[3] can react either with compound (2) to give draco-oxepine (4), or with water to afford the arylacetaldehyde (10), which can then condense with one or two flavan units (2) to yield the secobiflavanoid (3) or dracoflavan A (5), respectively (Scheme 2).

Experimental

M.p.s were measured in a Kofler apparatus and are uncorrected. UV spectra were measured for solutions in 95% EtOH on a JASCO Uvidec-510 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 177 instrument. Flash chromatography was performed with Merck silica gel (0.04–0.063 mm) and TLC with Merck HF_{254} silica gel. Mass spectra were taken on a VG-ZAB2 instrument at 70 eV. ^1H NMR spectra were recorded on a Bruker CPX-300 (300.13 MHz) spectrometer and ^{13}C NMR spectra on a Bruker AC 250L (62.9 MHz) instrument, with Me_4Si as internal standard. NOE difference spectra were obtained by subtracting alternatively right-off resonance-free induction decays (FIDs) from right-on resonance-induced FIDs.

Isolation and Purification of Dracoflavan A (5).—Finely powdered 'Dragon's blood' resin (100 g) was extracted in a Soxhlet apparatus first with hexane, and then with ethyl acetate. The hexane extract contained mainly flavans (1) and (2)[4] and terpenoid compounds,[9] while the EtOAc extract, after flash chromatography with hexane–EtOAc (2:1) as eluant, afforded compound (5), which was detected on TLC plates (Bakerflex IB-2F) by spraying with cerium(IV) in H_2SO_4 (orange-brown colour on heating): R_f 0.3 in hexane–EtOAc (2:1) and 0.4 in CH_2Cl_2–MeOH (30:1). *Dracoflavan A* (5) (150 mg) was obtained as a white solid, m.p. 148–153 °C; $[\alpha]_D$ −52.5° (*c* 0.1 in $CHCl_3$) (Found: C, 73.9; H, 5.7. $C_{49}H_{46}O_{10}$ requires C, 74.04; H, 5.83%); ν_{max} (Nujol) 3 350 (OH) and 1 730 cm^{-1} (OCOPh); *m/z* 795 (M^+ + 1), 690 [(M^+ + 1) − 105], 538 (M^+ − 256), 522, 434, and 256. ^1H and ^{13}C NMR data are reported in Tables 1 and 2, respectively.

Dracoflavan A Triacetate (5a).—A solution of compound (5) (30 mg) in dry pyridine (0.3 ml) was treated with Ac_2O (0.5 ml) for 30 min at room temperature. The reaction mixture was then extracted with CH_2Cl_2 and the extract was washed successively with saturated aq. $NaHCO_3$, water, saturated aq. $KHSO_4$, and water, and finally dried (Na_2SO_4). Evaporation of the solvent gave the triacetate (5a), m.p. 130–132 °C; $[\alpha]_D$ −8.5° (*c* 0.1 in $CHCl_3$) (Found: C, 71.3; H, 5.5. $C_{55}H_{52}O_{13}$ requires C, 71.72; H, 5.69%); ν_{max} (Nujol) 1 755 (OAc) and 1 735 cm^{-1} (OCOPh); δ_H ([$^2H_6$]acetone) 8.10–6.90 (15 H, m, 3 × Ph), 6.51 (1 H, s, 6-H), 6.11 and 6.00 (2 × 1 H, s, 6″- and 6‴-H), 5.19 (1 H, t, *J* 7.5 Hz, 8-H), 4.35 (2 × 1 H, m, 2″- and 2‴-H), 3.73 and 3.68 (2 × 3 H, s, 15″- and 15‴-H_3), 3.45 (2 H, m, 7-H_2), 3.41 (3 H, s, 9-H_3), 2.45 (2 × 2 H, m, 4″- and 4‴-H_2), 2.24, 1.96, 1.96, and 1.67 (4 × 3 H, each s, 3 × OAc and 10-H_3), and 1.90 (2 × 2 H, m, 3″- and 3‴-H_2).

Acidic Degradation of Dracoflavan A (**5**).—Compound (**5**) (100 mg) was dissolved in a solution of conc. HCl in MeOH (1:2) (10 ml), and the solution was refluxed for 30 min under nitrogen. Evaporation of the solvent, extraction with EtOAc and preparative TLC (PLC) of the residue with hexane–EtOAc (2:1) as developer gave (2*S*)-5-methoxyflavan-7-ol (**2**), (38%), identified by direct comparison (TLC, MS, $[\alpha]_D$, NMR) with an authentic sample,[4] and 1-*O*-methyl-2-methylphloroglucinol (**6**) (12%), as a white solid, m.p. 110–115 °C; *m/z* 154 (M^+), 139, and 123; $\delta_H(CDCl_3)$ 6.03 and 5.99 (2 × 1 H, d, *J* 2.4 Hz, 4- and 6-H), 4.67 and 4.59 (2 × 1 H, br signals, 3- and 5-OH), 3.78 (3 H, s, 1-OMe), and 2.02 (3 H, s, 2-Me).

Alkaline Degradation of Dracoflavan A (**5**).—Compound (**5**), (50 mg) was dissolved in 2M-KOH in MeOH (5 ml) and the solution was heated for 30 min on a steam-bath. Dilution with water, acidification, extraction with EtOAc, and PLC with hexane–EtOAc (2:1) gave, as the main product, (2*S*)-8-(6-hydroxy-4-methoxy-5-methylbenzofuran-2-yl)-5-methoxyflavan-7-ol (**7**), identified by direct comparison (MP, TLC, MS, NMR) with an authentic sample.[5]

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