CONSTITUENTS OF DRAGON'S BLOOD. PART III.[1] DRACOOXEPINE, A NOVEL TYPE OF BIFLAVANOID

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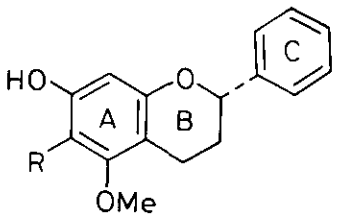
Abstract - From "Dragon's blood" resin (from Daemonorops draco Blume) a new biflavanoid with an unusual benzodioxepine moiety was isolated. Its structure was established by chemical and spectroscopic means. A possible mechanism of formation in the resin is proposed.

Many plants of different families produce red resins or exudates. Although they have been known for a long time in folklore or used in popular medicine, their chemical investigation started only recently, due to the complexity of their constituents.

Dragon's blood resin was formerly used in popular medicine against dysentery and as an astringent, whereas its present use is mostly as a varnish for musical instruments. It is most probably obtained from fruits of Daemonorops draco Blume (Palmae) in South East Asia. It was investigated first by the schools of Brockmann[2] and Robertson and Whalley[3], who elucidated the structures of the red flavonoid pigments dracorhodin and dracorubin.

Investigation of the resin showed the presence of a number of other constituents[1,4-7]: particularly significant was the isolation of the two flavans (1) and (2), as they appear now to be the precursors of the whole series of biflavanoids so far found in the resin, via various oxidative processes[1,5].



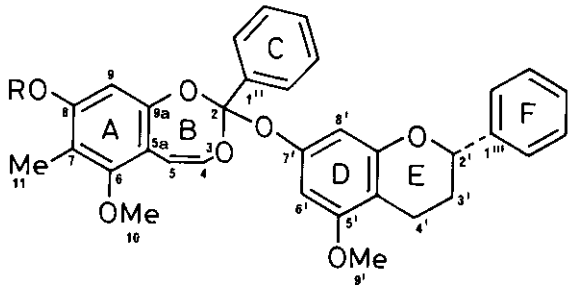
1 **R = H**
2 **R = Me**

A further example of the variety of the oxidation pathways in the resin is shown by the isolation of the compound (3) for which we propose the name dracooxepine. This paper deals with the structural elucidation of this compound.

Dracooxepine was obtained as white crystals, mp 103-105°C, $[\alpha]_{578}^{20}$ = +1,4° (c 0,2; $CHCl_3$); uv spectrum λ_{max} 224, 271, and 302sh nm (ε 55,300, 13,600 and 2,200). The mass spectrum indicated the formula $C_{33}H_{30}O_7$ (M^+, m/z 538) suggesting a biflavonoid structure, while the loss of the fragment with m/z 256 supported the presence of a substituted methoxyflavanol moiety[1]. This was confirmed by treatment of (3) with diluted HCl which afforded a crystalline compound, mp 87° C,

that was shown to be identical with (2S)-5-methoxyflavan-7-ol (1)[4].

The presence of one phenolic hydroxy group in the molecule was provided by the formation of the monoacetate (**3a**) (M^+, m/z 580; $C_{35}H_{32}O_8$) and the monomethyl ether (**3b**) (M^+, m/z 552; $C_{34}H_{32}O_7$). The ^1H nmr spectrum of (**3a**) (Table 1) showed the expected resonances for the flavan portion and



3 R = H
3a R = $^{12}CO^{13}Me$
3b R = Me

Table 1 - ^1H Nmr data for compound 3a in $CDCl_3$

Proton	δ/ppm[a,b]	J(H,H)/Hz		Proton	δ/ppm[a,b]	J(H,H)/Hz	
4	6.51	4,5	8.0	3'α	1.92	3'α,4'ß	10.5
5	5.99	5,9	0.6	3'ß	2.09	3'ß,4'α	3.4
9	6.71	9,11	0.5	4'α	2.61	3'ß,4'ß	6.0
10	3.69	2'ß,3'α	10.2	4'ß	2.52	4'α,4'ß	17.0
11	2.06	2'ß,3'ß	2.5	6'	5.82	6',8'	2.2
13	2.27	3'α,3'ß	13.7	8'	6.11	6',9'	0.3
2'ß	4.87	3'α, 4'α	6.3	9'	3.54		

[a] H-2" and H-6" resonate at 7.70 ppm and the remaining eight aromatic protons between 7.2 and 7.4 ppm.

[b] The 11, 13, 2', 3'α , 6' 8' and 9' protons present each two signals, attributable to two diastereoisomers (see later), which differ between 0.001 and 0.010 ppm.

signals attributable to the protons of a pentasubstituted (A) and a monosubstituted (C) aromatic rings, to two protons on a Z-1,2-disubstituted alkene ($J_{4,5}$ = 8.0 Hz), and to one OMe, one OAc and one aromatic methyl group, resonating at δ 3.69, 2.27 and 2.06 respectively.

Table 2. - ^{13}C Nmr data for compound **3a** in $CDCl_3$

Carbon[a]	δ/ppm[b,c]	^1J(C,H)/Hz	$>^1J$(C,H)/Hz
2	113.11	Sddd	8.5(H-4), ca. 4(H-2"), ca. 4(H-6")
4	139.82	Dd	193	4.5(H-5)
5	100.94	Dd	160	9.5(H-4)
5a	120.23	Sbrdd	9.5(H-4), 5.0(H-9)
6	155.28	Sdqq	4.0(H-5), 4.0(H_3-10), 4.0(H_3-11)
7	119.39	Sdq	5.5(H-9), 6.0(H_3-11)
8	148.49	Sdq	5.0(H-9), 4.5(H_3-11)
9	111.16	Ds	165	
9a	151.28	Sbrdd	6.5 (H-5), 5.5(H-9)
10	61.15	Qs	145	
11	9.42	Qd	129	1.5(H-9)
12	168.72	Sq	6.8(H_3-12)
13	20.76	Qs	130.5	
2'	77.51	Dm	144	
3'	29.27	Tm	130	
4'	19.24	Tm	131.5	
4'a	106.22	Sm	
5'	157.56	Sm	
6'	95.90	Dd	162	4.5(H-8')
7'	151.97	Sdd	4.5(H-6'), 4.5(H-8')
8'	101.55	Dd	164.5	4.5(H-6')
8'a	155.53	Sm	
9'	55.28	Qs	145	

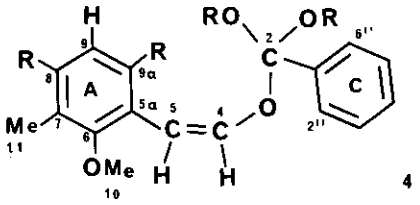
[a]The aromatic carbons 1", 2" and 6", 3" and 5", 4" resonate at δ 137.42, 126.88, 128.42, 129.51 and 1"', 2"' and 6"', 3"' and 5"', 4"' at δ 141.56, 125.99, 128.12, 127.75.

[b]Capital letters refer to the pattern resulting from directly bonded (C,H) couplings and small letters to that from (C,H) couplings over more than one-bond. S or s = singlet, D or d = doublet, T = triplet, Q or q = quartet, m = multiplet, and br = broad.

[c]The 2, 2', 3', 4', 4'a, 5', 6', 8', 8'a, 9', 1", 1"', 2"' and 6"' carbons present each two signals, attributable to two diastereoisomers (see later), which differ between 0.02 and 0.07 ppm.

The ^{13}C nmr spectrum confirmed and extended these findings through the appearance of 31 signals, four of them (C-2" and C-6", C-3" and C-5", C-2"' and C-6"', C-3"' and C-5"') were clearly of *twice the intensity of the others, thus giving 35 carbons in the molecule.* Chemical shift criteria and analysis of ^{1}H-^{13}C coupling constants as corroborated by ^{13}C-$\{^{1}H\}$ low-power specific decouplings and by one-bond ^{13}C-^{1}H and long-range ^{13}C-^{1}H shift correlated 2D nmr spectra (COLOC)[8] permitted their assignment (Table 2).

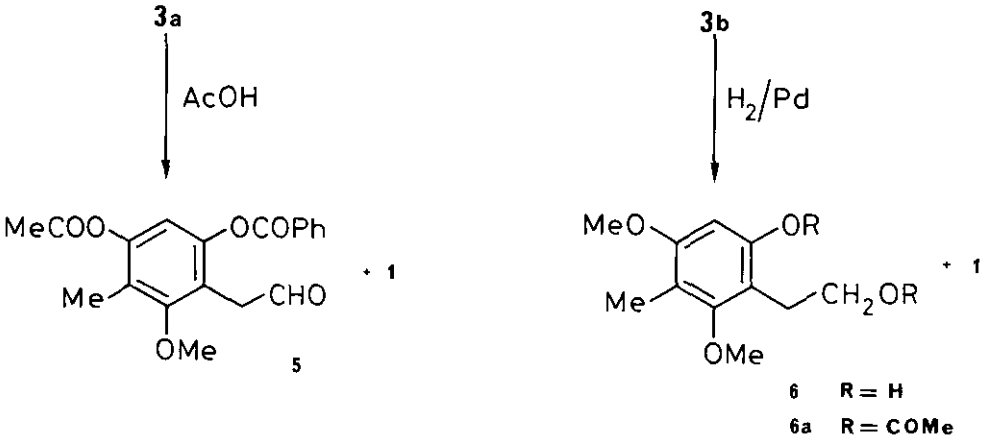
From the above spectral data the following partial structure (**4**) could be constituted.



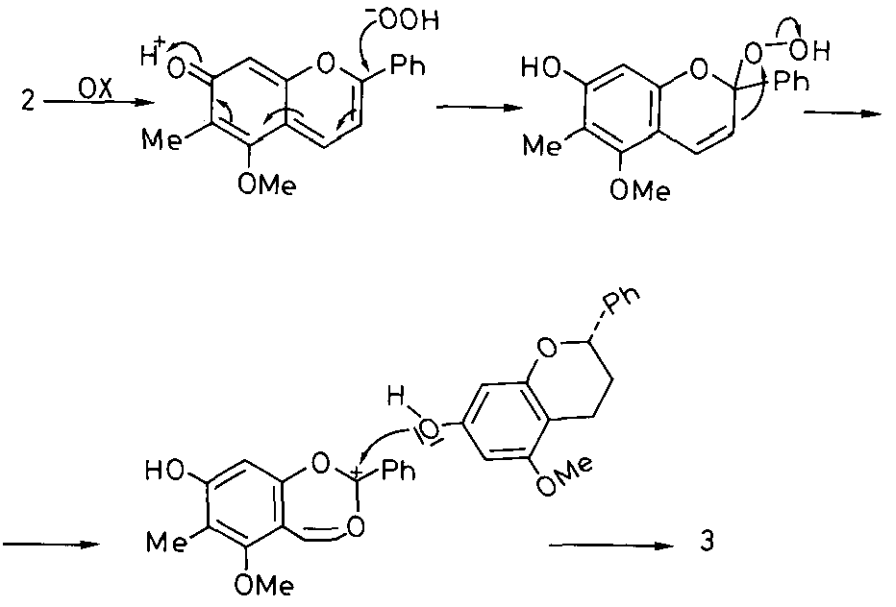
The substitution of ring A could be established as follows. The carbon bearing the OMe group (C-6) was identified as the one resonating at 155.28 ppm by selective irradiation of the O-methyl protons, H_3-10, which simplified the complex pattern of the C-6 signal to a quartet of doublets. The remaining three-bond couplings of 4 Hz were removed by irradiation of the 11-methyl protons and of the vinylic proton, H-5, resonating at 5.99 ppm. This fact, coupled with the observation of the presence of NOEs between H_3-10 and H_3-11 (2.5%) and H_3-10 and H-5 (9%), led us to place the *C-11 methyl group at C-7 and the C(5)=C(4) double bond at C-5a. The single proton of the ring* could only be allocated at C-9, since it presented three-bond couplings of 5.0 and 5.5 Hz with the meta-disposed C-5a and C-7. The signals at 148.49 and 151.28 ppm were assigned to C-8 and C-9a since they presented three-bond couplings with H_3-11 and H-5, respectively, and were both coupled to H-9. The OAc group must then be placed at C-8 or C-9a as H-9 underwent a downfield shift of 0.30 ppm with respect to (**3**) upon acetylation.

Moreover the ^{13}C nmr spectrum of (**3a**) contained the signal of a quaternary carbon at 113.11 ppm, *C-2, which presented couplings of 8.5 Hz with H-4 and of 4 Hz with both H-2" and H-6". The* chemical shift value[9] suggests that it is a part of an ortho ester moiety as shown in (**4**), while the above (C,H) couplings not only indicate that this group is the single substituent on ring C, but also that, of the three possible OR groups of the ortho ester, one must be the O-C(4)=C(5)H-one. The oxygen atoms of the two remaining OR groups must then constitute the bridges between C-2 and C-7' of the flavan moiety (**1**) and between C-2 and C-9a or C-8. Inspection of the Dreiding model of (**4**) clearly indicates that it would be impossible to link C-2 and C-8, this fact *permitting us to attribute the structure (3) to dracooxepine.*

Confirmatory evidence was provided by the following reactions. AcOH hydrolysis of the monoacetate



(3a) and hydrogenolysis of the methyl ether (**3b**) gave the two key compounds (**5**) and (6) respectively, together with the flavan (1). The presence of the 4-acetoxy and 4-methoxy groups in (**5**) and (**6**), respectively, confirms that the only free OH group in (3) must be located at C-8. The formation of (**5**) and (6) can be explained if the starting compound (3) contains the ortho ester



Scheme

function shown; this formulation is completely consistent with the mass spectrum, which requires seven oxygen atoms, with the presence of a cis CH=CH(O) fragment, with the absence of C=O absorption in the ir spectrum, and with the chemical shift of the quaternary carbon (C-2) in the ^{13}C nmr spectrum[9].

A possible mechanism of formation of the unusual compound (3) is shown in the Scheme. This oxidative process, which must have occurred in the resin, follows the pathway already proposed by Jurd[10] in 1966 for the H_2O_2 oxidation of flavylium salts, where a structure similar to (3) was postulated as an intermediate. In our case, the dioxepinium ion is trapped by the other flavan unit. The dissymmetric dimeric structure of (3) can be explained on the basis of the ascertained easier oxidation of the methyl-substituted flavan (2) with respect to the nor-compound (1)[1]. The mechanism would also require a non-stereospecific formation of the asymmetric center at C-2. Although we have not been able to see more than one peak in the hplc of (3), the fine splitting of some signals in the ^1H and ^{13}C nmr spectra of (3) lends support to the hypothesis that (3) is indeed a mixture of two diastereoisomers.

EXPERIMENTAL

All melting points are uncorrected. Uv spectra were measured for solutions in 95% EtOH on a JASCO Uvidec 510 spectrophotometer; ir spectra were taken with a Perkin-Elmer 177 instrument. Nmr spectra were recorded on a Bruker CXP-300 spectrometer using TMS as an internal standard; ms were measured with a VG-ZAB2 mass spectrometer.

Isolation procedure---We have already reported the extraction procedure from the resin to obtain several flavanoid products[1,4]; the isolation of the minor compound (3) was carried out during the same chromatographic separation. Compound (3) was detected on tlc plates (Bakerflex IB-2F) by spraying with cerium(IV) in sulphuric acid (red colour on heating); Rf 0.3 in hexane- EtOAc (3:1) and 0.7 in CH_2Cl_2 -MeOH (30:1), respectively.

Dracooxepine (3)---Mp 103-105°C, $[\alpha]_{578}^{20}$ = +1.4° (c 0.2; $CHCl_3$); (Found: C, 73.4; H, 5.7; $C_{33}H_{30}O_7$ requires C, 73.6; H, 5.6%), ms (m/z): 538 (M^+), 360, 283.0993 (calcd for $C_{17}H_{15}O_4$ 283.0970 ± 0.002), 256.1095 (calcd for $C_{16}H_{16}O_3$, 256.1099 ± 0.003), and 152. ^1H-Nmr(δ,$CDCl_3$): 7.72 (2H, m, H-2" and H-6"), 7.2-7.5 (8H, m, Ph), 6.44 (1H, d, J=8.0 Hz, H-4), 6.41 (1H, br s, H-9), 6.12 (1H, d, J=2.2 Hz, H-8'), 5.96 (1H, d, J=8.0 Hz, H-5), 5.86 (1H, br d, J=2.2 Hz, H-6'), 4.90 (1H, br dd, J=9.5 and 2.5 Hz, H-2'), 3.69 (3H, s, H_3-10), 3.55 (3H, br s, H_3-9'), 2.9-1.8 (4H, m, H_2-3' and H_2-4'), and 2.13 (3H, br s, H_3-11).

Monoacetyldracooxepine (3a)---Acetylation (Ac_2O-pyridine) of compound 3 gave the monoacetate **3a**, mp 104-107°C; $[\alpha]_D^{20}$ = -4.7° (C 0.2; $CHCl_3$); (Found: C, 72.2; H, 5.7; $C_{35}H_{32}O_8$ requires C, 72.4; H, 5.6%); ir (KBr) (ν max cm^{-1}): 1770 (Ac band), 1640, and 1600; uv (λ max, nm): 266 and 299 sh (ε 12,400 and 1,200); ms(m/z): 580 (M, 14%), 325(100), 283(64), 256(18), and 105(36), ^1H and ^{13}C-nmr data are collected in Tables 1 and 2 respectively.

Monomethyldracooxepine (3b)---Methylation (MeI, K_2CO_3, acetone) of compound 3 gave the monomethyl ether **3b**, mp 83-85°C; (Found: C, 73.6; H, 5.7; $C_{34}H_{32}O_7$ requires C, 73.9; H, 5.8%); uv (λ max, nm): 272, 280sh, and 300sh (ε 10,000, 8,600, and 1,500); ms(m/z): 552(M^+), 360, 343, 297, 281, 270, and 256.

Hydrolysis of compound (**3a**)---(**3a**)(50 mg) was kept with AcOH (3 ml) at 70°C for 2 h. Evapn of the solvent gave, after preparative tlc (hexane EtOAc, 2:1), two main compounds 1 and **5**; the (2S)-flavan **1** was identified by direct comparison with an authentic sample[4]; $[\alpha]_D^{20}$ = -6.2° (lit.[4] -6.3°). The same compound 1 could be isolated when dracooxepine **3** was treated with a mixture of MeOH-HCl (9:1) for 20 h at room temperature. **5**, viscous oil, has ir ($CHCl_3$)(ν max cm^{-1}): 1770(Ac), 1740 and 1730 (ArCOO- and RCHO); ms(m/z): 342(M^+), 324(M^+-18), 282(324-Ac), 267, 220, 205, 191, 178, and 177(282-COPh). ^{1}H-Nmr (δ ,$CDCl_3$): 9.66 (1H, t, J = 3 Hz, CHO), 8.13 and 7.54 (5H, m, $PhCO_2$), 6.71 (1H, br s, ArH), 3.74 (3H, s, OMe), 3.64 (2H, d, J=3Hz, CH_2), 2.33 (3H, s, OAc), and 2.16 (3H, br s, ArMe).

Hydrogenolysis of compound (**3b**)---(**3b**)(50 mg), dissolved in EtOAc-MeOH (1:1) (5 ml) was reduced for 20 h with 10% Pd on $BaSO_4$ (25 mg) to obtain compound **6** together with the flavan **1**; **6** was successively isolated (Ac_2O - Pyridine) as the acetate **6a**.

(**6**) M/z: 212(M^+), 194(M^+ - 18), 181, 165, 151, and 136.

^{1}H-Nmr (δ , $CDCl_3$): 6.34 (1H, br s, ArH), 3.97 (2H, m, CH_2OH), 3.78 and 3.66 (6H, s, 2 OMe), 2.92 (2H, m, $ArCH_2$), and 2.08 (3H, br s, ArMe).

(**6a**) Oil, ms(m/z): 254 (M^+); ^{1}H-nmr(δ , $CDCl_3$): 6.39 (1H, br s, ArH), 4.17 (2H, m, CH_2OH), 3.78 and 3.73 (6H, s, 2OMe), 2.82 (2H, m, $ArCH_2$), 2.33 and 2.03 (6H, s, 2 OAc), and 2.12 (3H, br s, ArMe).

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