203. A New Pyranocoumarin Diester from *Polygala paniculata* L.

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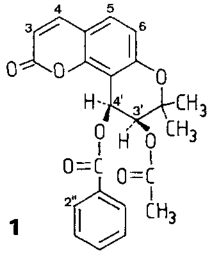
Summary

A new coumarin diester has been isolated from *Polygala paniculata* L. *(Polygalaceae)* by a combination of flash chromatography on silica gel and preparative reversed-phase chromatography. Its structure has been determined as 3'-*O*-acetyl-4'-*O*-benzoylkhellactone (= *9-acetoxy-9,10-dihydro-8,8-dimethyl-2-oxo-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*']dipyran-10-yl benzoate*) by spectroscopic methods (UV, IR, ^{1}H-NMR, EI- and CI-MS) and by X-ray analysis.

Introduction. – Plants of the genus *Polygala* are currently used in African and American folk medicine for the treatment of parasitical diseases [1] [2]. Recently, the molluscicidal activity of *Polygala erioptera* DC. has been reported [3]. A screening of several *Polygala* species for biologically active compounds revealed antifungal properties of the lipophilic extracts of *P. paniculata* L., as well as molluscicidal activity against *Biomphalaria glabrata* snails, the intermediate host of *Schistosoma mansoni*.

Several coumarins were isolated from the active petroleum-ether and $CHCl_3$-extracts. We now report the isolation and structure determination of the new dihydropyranocoumarin diester **1**.



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Results. – The structure of the new coumarin diester (for isolation, see *Exper. Part*) was elucidated by spectroscopic methods. The UV spectrum (MeOH) of the compound showed two major absorption maxima at λ_{max} 230 and 322 nm, which remained unchanged upon addition of NaOMe. In the IR spectrum, two absorption bands appeared in the carbonyl region at 1750 and 1730 cm^{-1}, together with bands for an aromatic ring. These data suggested, in addition to the strong blue fluorescence under *UV 366* light, the presence of a coumarin nucleus possessing no free phenolic OH-groups [4]. The ^1H-NMR spectrum confirmed this hypothesis. Two doublets at 6.24 (J = 9.5 Hz) and 6.90 ppm (J = 8 Hz) were typical for the protons at C(3) and C(6) of a coumarin [5]. The doublets of H–C(4) and H–C(5) at 7.66 (J = 9.5 Hz) and 7.45 ppm (J = 8 Hz), respectively, hidden in a complex pattern of aromatic protons, were attributed by spin-decoupling experiments. A pair of doublets at 5.45 and 6.88 ppm (J = 5 Hz), together with two CH_3 singlets at 1.48 and 1.60 ppm, indicated the presence of a heterocyclic ring fused to the benzopyrone system, most probably a dihydropyrano-diester [5]. The ester moieties were identified with the aid of the mass spectra. In the EI-MS, a weak molecular ion at *m/z* 408 could be observed. The presence of an Ac-group was indicated by a signal at *m/z* 348 ($[M-60]^+$), corresponding to the elimination of AcOH. This could be confirmed by the CH_3 singlet at 2.06 ppm in the ^1H-NMR spectrum. The fragment at *m/z* 287 ($[M-121]^+$) suggested a benzoyl group as a second ester moiety. This corroborated well with the presence of 5 aromatic protons in the ^1H-NMR spectrum between 7.45 and 8.09 ppm. Supplementary confirmation of the nature of the acyl residues was obtained by the intense signals in the EI-MS at *m/z* 105 ($[C_6H_5CO]^+$), 77 ($[C_6H_5]^+$) and 43 ($[CH_3CO]^+$), as well as by the CI-MS.

Diesters of dihydrofurano- and dihydropyranocoumarins show similar spectral data. An unambiguous structure elucidation of the compound was not possible on the basis of ^1H-NMR and mass spectral evidence only. The nature of the heterocyclic ring, the positions of attachment of the two acyl moieties and their relative configuration were determined by X-ray analysis. The structure was found to be 3′-*O*-acetyl-4′-*O*-benzoylkhellactone. The dihydropyranoside ring has a half-chair conformation with the twofold axis bisecting bonds C(7)–C(8) and C(12)–C(13). The two acid substituents at C(13) and C(14) are *cis* with torsion angle O(18)–C(13)–C(14)–O(22) 48.5°. The coumarin half of the molecule is planar within experimental error. Final positional parameters, bond distances, and angles are listed in *Tables 1* and *2*, respectively.

Table 1. *Final Positional Parameters* (× 10^4) *and Equivalent Isotropic Thermal Parameters* (× 10^4) *with e.s.d.'s in Parentheses*

$$(U_{eq} = \frac{1}{3}\Sigma_i\Sigma_j U_{ij}\, a_i^* a_j^* \bar{a}_i \cdot \bar{a}_j)$$

Atom	*x/a*	*y/b*	*z/c*	U_{eq}	Atom	*x/a*	*y/b*	*z/c*	U_{eq}
O(1)	3798 (5)	3542 (3)	4470 (3)	772 (17)	C(16)	7782 (8)	4513 (5)	7582 (4)	899 (28)
C(2)	3149 (12)	3211 (5)	3741 (5)	895 (33)	C(17)	6691 (7)	5732 (4)	6617 (4)	810 (25)
C(3)	4191 (14)	2967 (6)	3115 (6)	1080 (39)	O(18)	4600 (5)	4442 (3)	7455 (3)	791 (16)
C(4)	5643 (11)	3005 (5)	3196 (5)	934 (33)	C(19)	3795 (9)	3830 (5)	7897 (4)	887 (29)
C(5)	7776 (9)	3420 (4)	4105 (5)	808 (30)	O(20)	3829 (6)	2976 (3)	7787 (3)	1159 (23)
C(6)	8301 (8)	3735 (4)	4839 (5)	787 (27)	C(21)	2911 (10)	4329 (6)	8542 (5)	1199 (38)
C(7)	7275 (7)	3965 (4)	5466 (4)	654 (25)	O(22)	3987 (4)	5005 (2)	5878 (2)	655 (14)
C(8)	5777 (6)	3890 (4)	5323 (4)	599 (22)	C(23)	2562 (7)	5081 (5)	6075 (4)	729 (25)
C(9)	5318 (8)	3582 (4)	4574 (5)	666 (25)	O(24)	1817 (5)	4439 (4)	6314 (4)	1061 (21)
C(10)	6278 (9)	3334 (4)	3944 (4)	750 (27)	C(25)	2028 (7)	6078 (4)	5951 (4)	655 (24)
O(11)	7880 (4)	4235 (3)	6174 (3)	760 (17)	C(26)	2952 (7)	6869 (5)	5875 (4)	771 (26)
C(12)	6934 (7)	4648 (4)	6808 (4)	742 (24)	C(27)	2396 (8)	7785 (5)	5771 (4)	842 (28)
C(13)	5510 (6)	4066 (4)	6806 (4)	679 (22)	C(28)	901 (9)	7927 (6)	5748 (4)	902 (30)
C(14)	4692 (6)	4070 (4)	6020 (4)	658 (21)	C(29)	−55 (8)	7155 (6)	5837 (5)	955 (31)
O(15)	1816 (7)	3189 (4)	3733 (4)	1126 (25)	C(30)	512 (7)	6237 (5)	5935 (4)	840 (28)

Table 2. *Bond Distances* [Å] *and Angles* [°]

Distances [Å]				Angles [°]			
O(1)–C(2)	1.411 (10)	C(12)–C(17)	1.551 (8)	C(2)–O(1)–C(9)	122.0 (6)	C(13)–C(12)–C(17)	113.1 (5)
O(1)–C(9)	1.389 (8)	C(13)–C(14)	1.489 (9)	O(1)–C(2)–C(3)	114.2 (9)	C(16)–C(12)–C(17)	111.5 (5)
C(2)–C(3)	1.435 (14)	C(13)–O(18)	1.444 (7)	O(1)–C(2)–O(15)	115.7 (8)	C(12)–C(13)–C(14)	115.0 (5)
C(2)–O(15)	1.208 (13)	C(14)–O(22)	1.463 (6)	C(3)–C(2)–O(15)	130.1 (9)	C(12)–C(13)–O(18)	107.0 (5)
C(3)–C(4)	1.323 (16)	O(18)–C(19)	1.334 (8)	C(2)–C(3)–C(4)	125.0 (9)	C(14)–C(13)–O(18)	110.7 (5)
C(4)–C(10)	1.430 (11)	C(19)–O(20)	1.197 (8)	C(3)–C(4)–C(10)	119.9 (8)	C(8)–C(14)–C(13)	109.2 (5)
C(5)–C(6)	1.367 (11)	C(19)–C(21)	1.498 (11)	C(6)–C(5)–C(10)	122.4 (7)	C(8)–C(14)–O(22)	107.8 (4)
C(5)–C(10)	1.389 (12)	O(22)–C(23)	1.335 (7)	C(5)–C(6)–C(7)	118.8 (7)	C(13)–C(14)–O(22)	111.0 (4)
C(6)–C(7)	1.424 (10)	C(23)–O(24)	1.184 (8)	C(6)–C(7)–C(8)	120.1 (6)	C(13)–O(18)–C(19)	118.9 (5)
C(7)–C(8)	1.381 (9)	C(23)–C(25)	1.478 (9)	C(6)–C(7)–O(11)	115.0 (6)	O(18)–C(19)–O(22)	122.2 (7)
C(7)–O(11)	1.338 (8)	C(25)–C(26)	1.385 (9)	C(8)–C(7)–O(11)	124.9 (6)	O(18)–C(19)–C(21)	112.5 (6)
C(8)–C(9)	1.367 (10)	C(26)–C(27)	1.376 (10)	C(7)–C(8)–C(9)	118.4 (6)	O(20)–C(19)–C(21)	125.5 (7)
C(8)–C(14)	1.529 (9)	C(27)–C(28)	1.369 (11)	C(7)–C(8)–C(14)	119.5 (6)	C(14)–O(22)–C(23)	116.9 (5)
C(9)–C(10)	1.394 (10)	C(28)–C(29)	1.384 (11)	C(9)–C(8)–C(14)	121.9 (5)	O(22)–C(23)–O(24)	124.9 (6)
O(11)–C(12)	1.466 (8)	C(29)–C(30)	1.381 (11)	O(1)–C(9)–C(8)	115.1 (6)	O(22)–C(23)–C(25)	110.9 (5)
C(12)–C(13)	1.521 (8)	C(30)–C(25)	1.391 (9)	O(1)–C(9)–C(10)	121.2 (6)	O(24)–C(23)–C(25)	124.1 (6)
C(12)–C(16)	1.497 (10)			C(8)–C(9)–C(10)	123.7 (6)	C(23)–C(25)–C(26)	123.6 (6)
				C(4)–C(10)–C(5)	125.8 (8)	C(23)–C(25)–C(30)	118.3 (6)
				C(4)–C(10)–C(9)	117.7 (8)	C(26)–C(25)–C(30)	118.0 (6)
				C(5)–C(10)–C(9)	116.5 (7)	C(25)–C(26)–C(27)	121.3 (6)
				C(7)–O(11)–C(12)	119.1 (5)	C(26)–C(27)–C(28)	119.8 (7)
				O(11)–C(12)–C(13)	106.7 (5)	C(27)–C(28)–C(29)	120.3 (7)
				O(11)–C(12)–C(16)	104.8 (5)	C(28)–C(29)–C(30)	119.5 (7)
				C(13)–C(12)–C(16)	111.8 (5)	C(25)–C(30)–C(29)	121.0 (6)
				O(11)–C(12)–C(17)	108.5 (5)		

Discussion. – The new pyranocoumarin diester **1** has been isolated from *Polygala paniculata* L. A related dihydrofuranocoumarin with the same acid moieties, namely 4'-benzoyloxycolumbianetin acetate, has been previously described [6], but diesters with aromatic acids have rarely been found [7]. This is the first report on the occurrence of coumarins in the family *Polygalaceae*, and to our knowledge, the first description of a khellactone diester with an aromatic-acid moiety. Other coumarins from the same plant and their biological activity will be reported elsewhere [8].

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Experimental Part

General. Melting points (m.p.) were measured on a *Kofler* block and are uncorrected. UV spectra were recorded in MeOH on a *Perkin Elmer Lambda 3* spectrophotometer. IR spectra were obtained on a *Perkin Elmer 681* spectrometer. ^{1}H-NMR spectra were measured in $CDCl_3$ on a *Bruker WP 200* apparatus. Chemical shifts are given in δ (ppm) relative to TMS as internal standard. EI- and CI-(NH_3) mass spectra were obtained on a *Kratos MS-30* spectrometer.

Plant Material. The plant material was collected in Panama and a voucher specimen deposited at the University of Panama.

Extraction and Isolation. The dried plant (whole plant, 43 g) was extracted at r.t. successively with petroleum ether, $CHCl_3$, MeOH and MeOH/H_2O 1:1. Silica gel *60* (70–230 mesh, *Merck*) was used for column chromatography. Flash chromatography of the crude extract was performed with a 4 × 60 cm column (200 g silica gel); the gradient steps were: petroleum ether/AcOEt 70:30, 50:50, 25:75, followed by AcOEt/MeOH 99:1

and 98:2. The flow rate was 50 ml min^{-1}. For prep. reversed-phase chromatography, a *Lobar Lichroprep RP-8* column (40–63 µm, I.D. 2.5 × 27 cm; *Merck*) equipped with a *Duramat 80* pump (*Chemie und Filter*, Regensdorf) and a *LKB Uvicord II* detector (detection at 275 nm) were used. TLC was carried out on silica-gel-precoated Al sheets *(Merck)* with petroleum ether/AcOEt 1:1, or on precoated *RP-8* nanoplates *(Merck)* and $MeOH/H_2O$ 8:2 as mobile phase.

*3'-*O*-Acetyl-4'-*O*-benzoylkhellactone (= (9*R**-cis)-9-(Acetoxy)-9,10-dihydro-8,8-dimethyl-2-oxo-2*H*,8*H*-benzo[1,2-*b*:3,4-*b'*]dipyran-10-yl Benzoate).* Colourless needles from heptane/AcOEt, m.p. 144–145°. TLC (SiO_2, petroleum ether/AcOEt 1:1): R_f 0.33, (*RP-8*, $MeOH/H_2O$ 8:2): R_f 0.40. UV (MeOH): 221 (22100), 254 sh (4600), 298 sh (8900), 332 (14500). IR (KBr): 1750, 1730, 1610, 1500. ^1H-NMR (200 MHz, $CDCl_3$): 8.09 (*m*, 2H, H–C(2''), H–C(6'')); 7.66 (*d*, J = 9.5, 1H, H–C(4)); 7.61 (*dt*, J = 8 and 2, 1H, H–C(4'')); 7.45–7.48 (*m*, 2H, H–C(3'') and H–C(5'')); 7.45 (*d*, J = 8, 1H, H–C(5)); 6.90 (*d*, J = 8, 1H, H–C(6)); 6.88 (*d*, J = 5, 1H, H–C(4')); 6.24 (*d*, J = 9.5, 1H, H–C(3)); 5.45 (*d*, J = 5, 1H, H–C(3')); 2.06 (*s*, 3H, CH_3COO); 1.60 (*s*, 3H, CH_3–C(2')); 1.48 (*s*, 3H, CH_3–C(2')). EI-MS (70 eV): 408 (1.4), 348 (3.8), 333 (20), 303 (0.7), 287 (0.7), 261 (2.4), 243 (3.5), 229 (7.7), 105 (100), 77 (33), 43 (28), CI-MS (NH_3): 426, 348, 333, 287 (base peak), 229, 105, 77.

X-Ray Analysis. Suitable crystals were grown from an Et_2O/heptane solution. Crystal data: $C_{23}H_{20}O_7$, M_r = 408, space group $P2_12_12_1$, a = 9.06 (2), b = 13.86 (2), c = 16.42 (1) Å, V = 2061.9 $Å^3$, Z = 4, D_c = 1.314 Mgm^{-3}, Mo$K\alpha$, λ = 0.7107 Å, μ = 0.6 cm^{-1}. Two crystals of similar dimensions (0.25 × 0.25 × 0.35 mm) were used for data collection. Preliminary *Weissenberg* and precession photographs indicated the crystals to be orthorhombic, space group $P2_12_12_1$. Intensity data for layers with h = 0–8 and k = 0–13 and with $\theta < 25°$, were measured on a *Stoe STADI-2* two-circle diffractometer (graphite-monochromated Mo$K\alpha$-radiation). 2799 independent reflections were measured using the variable ω-scan technique [9]; 1348 were considered observed [$I > 1.5\sigma(I)$]. The structure was solved by direct methods using the SHELX-76 system [10], which was also used for all further calculations. In the final cycles of least-squares refinement H-atoms were included at calculated positions (C–H 1.08 Å), with overall isotropic thermal parameters for the benzene-type and the primary and tertiary CH_3-type H-atoms (final values 0.11, 0.14, 0.07 $Å^2$, respectively). Weighted anisotropic blocked-matrix least-squares refinement for 1342 reflections (6 reflexions probably suffering from extinction were removed) converged at R = 0.062, R_w = 0.059, $w = 1/[\sigma^2|F_o| + 0.00070\,|F_o|^2]$. In the final cycle the average parameter shift was less than 0.3 e.s.d. A final difference map showed no significant residual density. Final positional and thermal parameters are given in *Table 1*. Bond distances and angles are given in *Table 2*. The numbering scheme used is apparent from the *Figure* prepared using ORTEP [11].

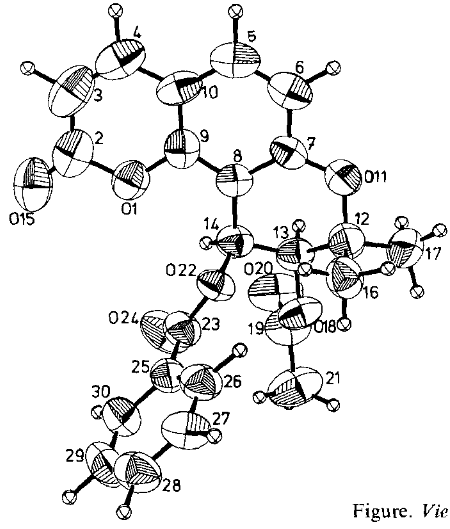

Figure. *View of the molecule* **1** *showing the atomic numbering and vibrational ellipsoids* (50% probability level)

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