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GENTIOLACTONE, A SECOIRIDOID DILACTONE FROM *GENTIANA PURPUREA*

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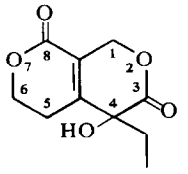
Key Word Index—*Gentiana purpurea*; Gentianaceae; secoiridoid; gentiolactone.

Abstract—The structure of gentiolactone, a new secoiridoid dilactone isolated from the dried roots of *Gentiana purpurea* was elucidated by spectroscopic, spectrometric and X-ray diffraction methods as (±)-4-ethyl-4-hydroxy-3,4,5,6-tetrahydro-1H,8H-pyrano[3,4-*c*]-pyran-3,8-dione.

INTRODUCTION

The isolation and characterization of several constituents of *Gentiana purpurea* have been reported [1–7]. During a search for phenolic compounds [8] in root material of this species, a compound which later proved to be a secoiridoid was isolated and its structure (**1**) elucidated by spectroscopic and spectrometric methods. The compound shows no optical activity and an X-ray diffraction analysis was undertaken to confirm the structure and show that the compound is racemic.



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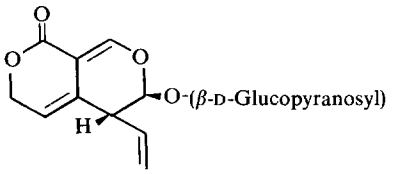

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RESULTS AND DISCUSSION

In the MS of **1** a very small peak at *m/e* 212 was found to represent the parent ion for both the *m/e* 183 ($C_8H_7O_5^+$) and *m/e* 168 ($C_9H_{12}O_3^+$) ions, thus it was a reasonable candidate for M^+. Its composition ($C_{10}H_{12}O_5^+$) was deduced from its fragmentations which proceed through loss of CO_2 and $C_2H_5\cdot$ by alternative routes leading to the base peak ion at *m/e* 139 ($C_7H_7O_3^+$).

The IR spectrum of **1** in KBr showed a remarkably broad and intense carbonyl band at 1725–1715 cm^{-1} which recorded in $CHCl_3$ was split to give 1725 and 1745 cm^{-1} bands. In conjunction with prominent bands in the C—O stretching region and the loss of CO_2 in the MS the presence of lactone groups were confirmed. Bands close to 3500 cm^{-1} (OH), and a weak band at 1600 cm^{-1} in the solution spectrum (>C=C< conjug.) also fit the structure.

In the PMR spectrum of **1** in $CDCl_3$, signals at δ 1.01 (3H, *t*) and at δ 1.5–2.1 (2H, *m*) were found by double resonance experiments to represent an ethyl group which was not further proton coupled. The non-equivalence of the methylene protons, which may be approximated as mutually displaced quartets was in agreement with positioning of the ethyl group at C-4, a chiral centre [9]. The presence of the hydroxyl group was confirmed by a signal at δ 3.6 (1H, *s*) which disappeared upon addition of D_2O. In DMSO-d_6 this signal was at δ 6.15 and was still a singlet indicating that the alcoholic function was tertiary [10].

The remaining signals in the PMR spectrum represented the ring methylene groups. A signal at δ 4.54 was a triplet (J = 6.5 Hz) while at δ 4.9–5.5 was seen an AB system of doublets (J = 16.5 Hz), each further split into triplets (J = 2.5 and 1.5). By irradiation at the frequency of a signal at δ 2.6–2.9 (*m*) the methylene triplet collapsed into a singlet while the triplet splitting of the other methylene protons disappeared. The low-field protons were those at C-6 and C-1, deshielded by the neighbouring oxygen atoms. The latter set were further deshielded by the double bond and are obviously magnetically non-equivalent. Their homoallylic coupling with the methylene protons at C-5 have constants of expected magnitude for the transoid case [11].

In the ^{13}C-NMR spectrum of **1** in $CDCl_3$ signals at δ 7.7 (*q*) and 22.7 (*t*) corresponded to the ethyl group. A signal at δ 31.0 (*t*) arose from the allylic methylene carbon C-5. A signal at δ 66.7 (*t*) corresponded to the methylene carbon atoms bonded to oxygen in the ester groupings; remarkably, they were isochronous although their protons were not. The carbonyl functions showed up at δ 172.6 (*s*) and 161.6 (*s*), in good agreement with the values expected for a six-membered lactone (*ca* δ 175) and for its α,β-unsaturated counterpart, since the latter may be expected to resonate at 5–15 ppm higher field in analogy with other carbonyl compounds [12]. The α and β carbon signals were seen at δ 120.0 (*s*) and 153.4 (*s*), respectively. A signal at δ 72.3 (*s*) was assigned to the carbon atom carrying the alcohol group.

The UV absorption was consistent with the chromo-

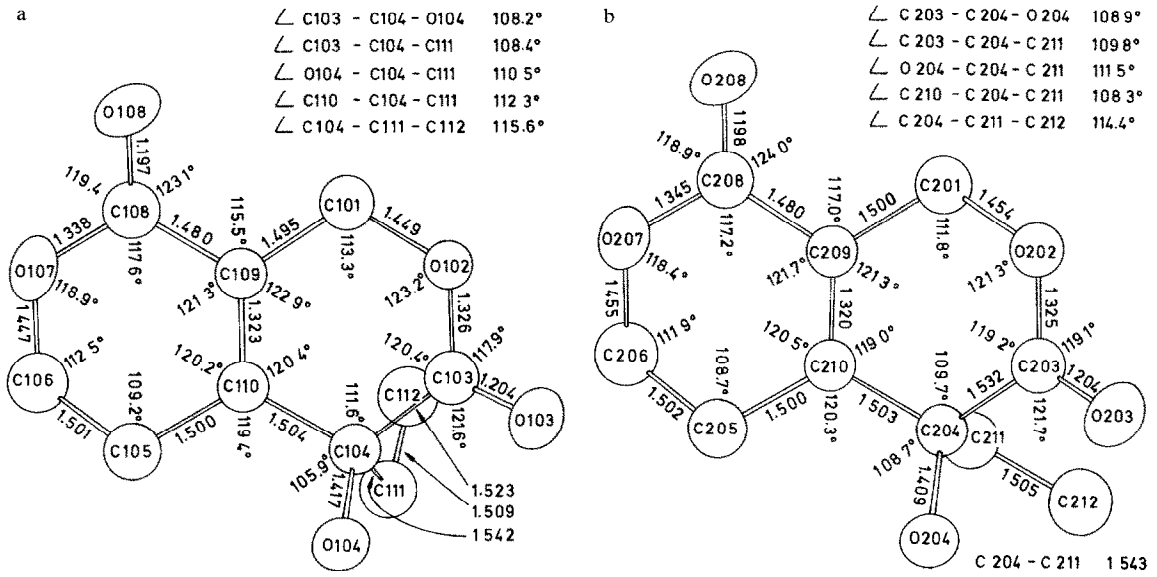

Fig. 1a and b. Bond lengths (Å), angles (°) and conformations of the two crystallographically independent molecules of gentiolactone. The estimated standard deviations on bond lengths and angles are 0 004–0.006 Å and 0.2–0.3 , respectively. The drawings were produced by ORTEP II [17].

phore present in **1**. α,β-Unsaturated acids and esters in which the double bond is fully substituted absorb at 225 ± 5 nm [13] and the same may be expected to hold true for lactones.

Table 1. Final positional parameters

Atom	x/A	y/B	z/C
C101	0.3296 (4)	0.9588 (3)	0.0729 (3)
O102	0.4586 (3)	0.9722 (2)	0.1598 (2)
C103	0.4409 (4)	0.9831 (3)	0.2836 (3)
C104	0.2799 (4)	1.0011 (3)	0.3360 (3)
C105	0.0041 (4)	0.9011 (3)	0.2977 (3)
C106	−0.1207 (5)	0.9043 (4)	0.2014 (4)
O107	−0.0773 (3)	0.8357 (3)	0.0833 (2)
C108	0.0648 (4)	0.8549 (3)	0.0413 (3)
C109	0.1801 (4)	0.9235 (3)	0.1282 (3)
C110	0.1558 (4)	0 9412 (3)	0.2418 (3)
O103	0.5557 (3)	0.9828 (3)	0.3442 (2)
O104	0.2739 (3)	0.9327 (3)	0.4392 (2)
O108	0.0944 (3)	0.8138 (3)	−0 0632 (2)
C111	0.2543 (4)	1.1475 (3)	0 3756 (4)
C112	0 2616 (4)	1 2316 (4)	0 2739 (3)
C201	0.8008(4)	0.5306 (3)	0.0875 (3)
O202	0.9337 (3)	0.5353 (3)	0.1710 (2)
C203	0.9167 (4)	0.5228 (3)	0.2887 (3)
C204	0.7544 (4)	0.4974 (3)	0.3361 (3)
C205	0.4899 (4)	0.6172 (3)	0 3330 (3)
C206	0.3608 (4)	0.6112 (4)	0.2389 (4)
O207	0.4039 (3)	0.6743 (3)	0.1336 (2)
C208	0.5417 (4)	0.6452 (3)	0.0813 (3)
C209	0.6566 (4)	0.5798 (3)	0.1526 (3)
C210	0 6358 (4)	0.5688 (3)	0.2697 (3)
O203	1.0293 (3)	0.5320 (3)	0.3557 (2)
O204	0.7498 (3)	0 5443 (2)	0.4626 (2)
O208	0 5659 (3)	0.6752 (3)	−0.0185 (2)
C211	0.7161 (4)	0 3508 (3)	0.3112 (3)
C212	0.8278 (5)	0.2661 (4)	0.3701 (4)
H104	0.338 (5)	0.961 (4)	0.488 (4)
H204	0.836 (5)	0.523 (4)	0.498 (3)

The X-ray diffraction analysis showed that the compound was racemic. The final positional parameters are listed in Table 1. The molecular dimensions and conformations of the two crystallographically independent molecules are given in Fig. 1a and b together with the numbering used for the non-hydrogen atoms. The one major difference between the two independent molecules was found for the torsion angle C*x*10–C*x*04–C*x*11–C*x*12, the value of which was ±63.4(4)° for $x = 1$ and ±178.8 (3)° for $x = 2$. The crystal packing is illustrated in Fig. 2. *Inter*-molecular hydrogen bonds O*x*04–H*x*04 ... O*x*03 link molecules related by a center of symmetry together in pairs. The dimensions of the hydrogen bonds are listed in Table 2 together with distances and angles for the *intra*-molecular contacts O*x*04–H*x*04 ... O*x*03. The crystal structure is stabilized by a great number of van der Waals' contacts.

The structure of gentiolactone includes the secoiridoid carbon skeleton, a taxonomic feature already associated with the Gentianaceae [14]. Gentiopicroside (**2**) a member of the same class is found in roots of *G. purpurea* [5]. The possibility that gentiolactone is formed from this compound may be considered. In that case the configurational identity of the side chain-bearing carbon atom is lost in the process.

Table 2. *Inter*-molecular hydrogen bonds and *intra*-molecular contacts O*x*04–H*x*04 . O*x*03. Distances (Å) and angles (°)

A–H ... B	A–H	A ... B	H ... B	∠AHB
O104–H104 .. $O103_{(1-x,\,2-y,\,1-z)}$	0.79 (4)	2.810 (4)	2.05 (4)	159 (4)
O204–H204 ... $O203_{(2-x\,\,1-y,\,1-z)}$	0.87 (4)	2.927 (4)	2.12 (4)	154 (4)
O104–H104 .. O103	0.79 (4)	2.728 (4)	2.49 (4)	99 (3)
O204–H204 ... O203	0.87 (4)	2.688 (4)	2.31 (4)	107 (3)

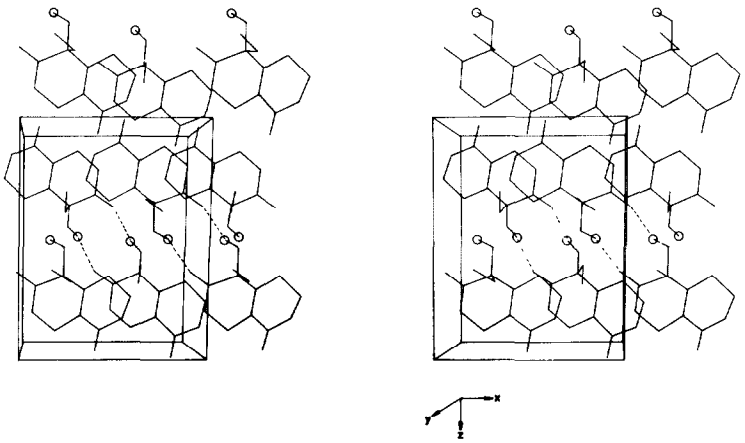

Fig. 2. Stereo view of the crystal packing. The hydroxyl hydrogen atoms are indicated by circles and hydrogen bonds by broken lines.

EXPERIMENTAL

Extraction and purification. Dried roots of *Gentiana purpurea* L. were available commercially from Caesar & Loretz, Hamburg. The powdered root material (1 kg) was defatted with petrol, then extracted with CH_2Cl_2 (Soxhlet) for about 70 hr. Evaporation afforded 15.8 g residue which was taken up in 90% MeOH. The soln was extracted with petrol and the MeOH phase evapd. Column chromatography on Si gel (700 g) in CH_2Cl_2 afforded **1** in the fractions eluted with CH_2Cl_2–EtOAc (99:1). Further purification was effected on a similar column eluted with C_6H_6–EtOAc mixtures, yielding 180 mg compound **1**. Crystallization from EtOH afforded colourless irregular prisms, mp 119–120.5° (corr.). TLC on Si gel F_{254}, $CHCl_3$–EtOAc (4:1) showed a single spot, R_f 0.21 in short wave UV light. With the NH_2OH–$FeCl_3$ spray reagent a weak colour reaction was also obtained. TLC on polyamide F_{254} (Merck Fertigplatten) using MeOH also showed a single spot, R_f 0.75. $[\alpha]_\lambda^{25}$ 0° (MeOH: 0.855) for λ between 365 and 589 nm. λ_{max}^{MeOH} nm log ε): 225–230 (3.74), decreasing with time upon addition of NaOMe, unaffected by HCl. λ_{max}^{MeCN} nm (log ε): 227 (3.75). ν_{max}^{KBr} cm^{-1}: 3490 *s*, 3460 *s*, 3020 *w*, 2980 *w*, 2940 *w*, 2920 *w*, 2850 *w*, 1725–1715 *vs*, 1470 *m*, 1445 *w*, 1425 *w*, 1410 *m*, 1375 *w*, 1350 *w*, 1325 *m*, 1310 *s*, 1295 *w*, 1260 *m*, 1230 *w*, 1220 *w*, 1205 *m*, 1180 *m*, 1170 *m*, 1155 *s*, 1135 *s*, 1110 *m*, 1065 *m*, 1040 *m*, 1020 *s*, 995 *m*, 965 *w*, 940 *w*, 920 *w*, 890 *w*, 815 *w*, 760 *m*, 720 *w*, 660 *w*, 625 *w*.

MS. (AEI-MS 902 instrument, probe) 70 eV, *m/e* (rel. int.): 212 (0.5), 184 (8), 183 (37), 182 (7), 169 (7), 168 (40), 156 (3), 155 (4), 150 (3), 140 (16), 139 (100), 138 (9), 137 (3), 125 (4), 124 (3), 123 (16), 122 (4), 121 (32), 112 (3), 111 (7), 109 (4), 108 (2), 98 (2), 97 (4), 95 (6), 93 (4), 91 (4), 83 (5), 82 (3), 81 (12), 80 (4), 79 (5), 77 (5), 69 (7), 68 (5), 67 (37), 66 (7), 65 (14), 63 (3), 57 (48), 56 (3), 55 (8), 53 (19), 52 (12), 51 (12), 50 (5). *m**, 134.0, 115.0 (168 → 139), 105.5 (183 → 139), 91.0, 90.1, 88.8, 84.7 and 63.1. Metastable defocusing: 212 → 168, 212 → 183 and 196 → 168. High resolution measurements: 183.0293, 168.0786 and 139.0395.

NMR. PMR spectra were recorded on a Bruker HX-90-E instrument. The ^{13}C NMR spectrum was recorded on a Bruker WH-90 instrument. Chemical shifts are relative to TMS as the internal standard.

X-ray analysis. The material used for the X-ray examination was recrystallized from $CHCl_3$ and single crystals were grown from a mixture of $CHCl_3$ and CCl_4 by slow evaporation at 5°.

Crystal data. 4-Ethyl-4-hydroxy-3,4,5,6-tetrahydro-1H,8H-pyrano[3,4-*c*]pyran-3,8-dione, $C_{10}H_{12}O_5$, M = 212.20. Triclinic, a = 8.614 (3), b = 10.382 (3), c = 10.973 (2) Å, α = 97.28 (2), β = 90.76 (3), γ = 90.35 (3)°. U = 972.2 Å^3. D_m (flotation) = 1.448 g cm^{-3}, Z = 4, D_c = 1.450 g cm^{-3}. Linear absorption coefficient for X-rays [λ (Mo$K\alpha$ = 0.7107 Å], μ = 1.25 cm^{-1}, $F(000)$ = 448. Space group $P\bar{1}$. Three-dimensional diffraction data were measured at room temp. on a Nonius three-circle automatic diffractometer using standard techniques. The structure was solved and refined using MULTAN [15] and programs of the X-RAY-system [16], respectively. Detailed information about experimental conditions and the refinements are, together with the final lists of structure factors, thermal parameters and calculated positional parameters for hydrogen atoms, available from the authors on request.

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