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ISOLATION AND SYNTHESIS OF 3-CHLOROGENTISYL ALCOHOL—A METABOLITE OF *PENICILLIUM CANADENSE*

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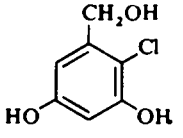
Abstract—A Minor metabolite of *Penicillium canadense* has been shown to be 3-chlorogentisyl alcohol by comparison with its isomer 2-chloro-3,5-dihydroxybenzyl alcohol and by synthesis.

IT IS WELL KNOWN that the UV spectra of hydroquinones show a hypsochromic shift upon basification (in contrast to isomeric dihydroxybenzenes). Less well known, but more striking, are the intense maxima at higher wavelengths which can be developed by hydroquinones under different conditions. This effect assisted in the structural elucidation of a new chlorine containing metabolite (**1**) isolated from culture filtrates of *Penicillium canadense*.

This metabolite crystallized from EtOAc–light petroleum in needles, m.p. 144°, $C_7H_7ClO_3$ (M^+ at 174·0075), whose three OH groups are present as two phenolic groups (purple reaction with $FeCl_3$, exchangeable 1H multiplets at 0·88 and 1·61 τ in the NMR using d_6-DMSO as solvent) and as part of a hydroxymethyl substituent on an aromatic ring (2H singlet at 5·48 τ and exchangeable 1H multiplet at 4·92 τ). The remaining two protons gave doublets (J = 3 Hz) at 3·35 and 3·21 τ, the latter being sharpened upon irradiation at the 2H singlet at 5·48 τ, indicating the presence of two *meta* aromatic protons, one of which is located *ortho* to a hydroxymethyl group. The 1, 2, 3, 5 substitution pattern indicated by the NMR data was also suggested by the pattern of weak overtone and combination bands between 1740 and 1950 cm^{-1} in the IR.[1] From these data, the metabolite would appear to be one of the three chlorodihydroxybenzyl alcohols **1**, **2** or **3**.

1



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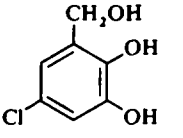


3

In keeping with this, the metabolite gave the triacetate **12** (acetylation shift of benzylic methylene group to 4·98 and of the aryl hydrogen doublets to 2·78 and 2·89 τ). Upon PLC on silica, hydrolysis occurred to give the more polar diacetate **13**. The phenolic nature of this compound was shown by its green reaction with $FeCl_3$ and its UV λ_{max} EtOH 315 nm (ε 2900) shifting to 348 nm (ε 3400) in base and the

position of the phenolic grouping by its IR ν_{max} 3500 and the chemical shift of the aryl protons (3·15 and 3·24 τ).

Since basification is known to produce hypsochromic shifts in the UV spectra of hydroquinones and bathochromic shifts in those of resorcinols or catechols,[2] the shift of the spectrum of the metabolite to higher wavelengths upon addition of alkali was at first taken to favour structures **2** or **3**. However, the metabolite gave a negative vanillin–*p*-TsOH test for a catechol[3] and structure **2** was eliminated by comparison of the metabolite with a sample of **2** synthesized from diacetyl-α-resorcylic acid (**4**).[4] This synthesis involved selective reduction of the carboxyl group of **4** with one equivalent of diborane in THF to give the benzyl alcohol **5** (ν_{max} 3520). The corresponding triacetate **6** slowly underwent monochlorination in HOAc to give **7**, which afforded **2** upon hydrolysis. The mass spectrum of **2**, lacked a strong $M^+ - H_2O$ ion, in contrast to that of the metabolite in which this ion was the base peak. This is in accord with these isomers being *meta*- and *ortho*-hydroxybenzyl alcohols respectively.[5]

The effect of base on the UV spectrum of the metabolite was in fact found to be almost identical to that of hydroquinone itself (Fig 1) indicating structure **1** for the

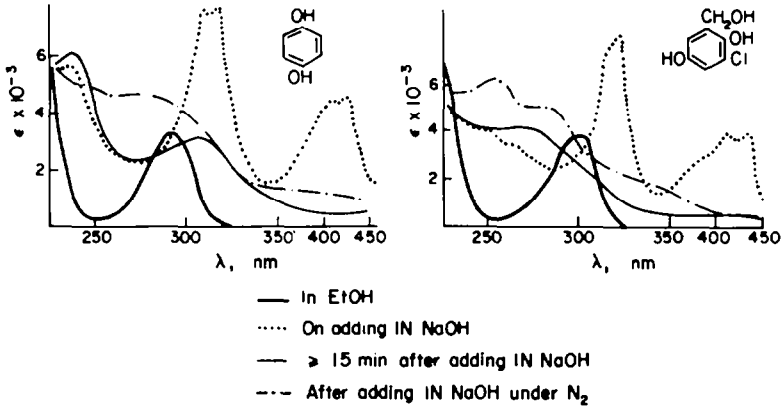
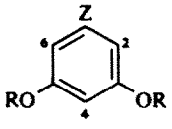


Fig 1. Base shifts in the UV spectra of hydroquinones

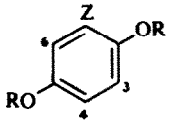
metabolite. Instead of the hyposochromic shift upon basification expected from casual examination of the literature,[2] a series of intense peaks (at 314, 320, 408 and 433 nm) immediately developed, although these gradually collapsed ($t_{\frac{1}{2}}$ *ca.* 2 min) to broad absorption centred at 267 nm (285 nm in the case of hydroquinone). From studies on 2,5-di t-butylhydroquinone[6] these peaks are evidently due to semiquinone ions formed by autoxidation of the hydroquinone. Normal hypsochromic shifts (15 nm for the metabolite and 10 nm for hydroquinone) were however observed when the spectrum was recorded under N_2 using deoxygenated NaOH aq as base.

Finally, the structure **1** of the metabolite was confirmed by synthesis in 32% yield from diacetylgentisic acid **8**.[7] In order to introduce chlorine into the required position, selective hydrolysis of the 2-acetate grouping was first carried out by refluxing **8** in dioxan-water at pH 6·0 to give **9** (blue reaction with $FeCl_3$, upfield shift of H-3 to 3·10 τ) and then chlorinating the corresponding methyl ester (**10**) using chlorine

TABLE 1. NMR DATA OF 3-CHLOROGENTISYL ALCOHOL AND RELATED PHENOLS AND ACETATES*



	Z	R	R	τH-2	τZ	τH-4	τH-6	τR	
4	CO_2H	Ac	Ac	2·67 (2·5)	0·03	3·13 (2·5)	2·67 (2·5)	7·80	
5	CH_2OH	Ac	Ac	3·06 (2)	5·19 5·80†	3·22 (2, 2)	3·06 (2)	7·76	
6	CH_2OAc	Ac	Ac	3·00 (2·0)	4·90 7·90	3·07 (2·0)	3·00 (2·0)	7·71	
7	CH_2OAc	Ac	Ac	Cl	4·73 7·82	2·98 (2·5)	2·83 (2·5)	7·70	7·77
2	CH_2OH	H	H	Cl	5·27	3·58 (2)	3·30 (2)	-	



	Z	R_1	R_2	τH-3	τZ	τH-4	τH-6	τR_1	τR_2
8	CO_2H	Ac	Ac	2·77 (m)		2·77 (m)	2·25 (2·5)	7·67	7·67
9	CO_2H	H	Ac	3·10 (9)		2·73 (3, 9)	2·36 (3)		7·73
10	CO_2Me	H	Ac	3·07 (9)	6·06	2·80 (3, 9)	2·25 (3)	- -	7·73
11	CO_2Me	H	Ac	Cl	6·03	2·67 (3)	2·47 (3)	- -	7·78
1	CH_2OH	H	H	Cl	5·48 4·98†	3·35 (3)	3·21 (3)	0·88†	1·61†
12	CH_2OAc	Ac	Ac	Cl	4·98 7·97	2·89 (3)	2·78 (3)	7·76	7·68
13	CH_2OAc	Ac	H	Cl	5·02 7·84	3·24 (3)	3·15 (3)	- -	7·68

* NMR spectra were run in $CDCl_3$ except for **2** (CH_3COCD_3) and **1** (CD_3SOCD_3). (Coupling constants given in parenthesis)

† 1H, s, exchangeable

water or Cl_2 in HOAc. The resulting chloroester (**11**) then afforded 3-chlorogentisyl alcohol (**1**) by reduction with an excess of LAH in ether. Chlorination of **10** in MeOH gave a yellow crystalline compound with the properties appropriate for carbomethoxytrichlorobenzoquinone.

Since completion of this work, 3-chlorogentisyl alcohol has been isolated independently from *Phyllosticta* species[8] and from a *Phoma* species[9] in both cases the orientation of substituents being the most biogenetically probable in view of the co-occurrences of gentisyl alcohol and a number of related compounds. Synthesis of **1** from 6-chloro-*o*-cresol (without characterization of intermediates),[8] and from chlorohydroquinone[9] (*ca* ½% yield in both cases) provided structural confirmation.

EXPERIMENTAL

R_fs refer to 0·25 mm layers of Kieselgel G, using *p*-aminoazobenzene and Sudan yellow as standards (R_f 0·51 and 0·62 respectively using $CHCl_3$ as eluant).

Isolation of 3-*chlorogentisyl alcohol* (**1**). The mould *Penicillium canadense* (C.M.I No. 95, 493) was grown on Czapek-Dox medium for 10 days in Roux bottles at 25°. The culture filtrate, after neutralization,

was stirred with activated charcoal [10g/l of broth] for 24 hr, and the adsorbed metabolites recovered by Soxhlet extraction with acetone for 24 hr. Chromatography on silicic acid allowed separation of the less polar metabolites from 3-*chlorogentisyl alcohol* (**1**) which was finally eluted with 5% EtOAc in $CHCl_3$ and crystallized as needles, m.p. 144° (EtOAc-light petroleum), R_f 0·25 (4% MeOH in $CHCl_3$), ν_{max} (KBr) cm^{-1}: 3425, 1597, 1455, 1302, 1160, 1105, 1028, 978, 848, 843, 792, λ_{max} (EtOH) 294 nm (ε 3910): λ_{max} (EtOH-NaOH aq) 262 nm (ε 3900), 314 (7060), 320 (8120), 408 (4140), 433 (4320): λ_{max} [EtOH-NaOH aq (N_2)] 280 nm (ε 5100). NMR (CD_3SOCD_3, 100 MHz): τ 5·48 (2H, s, $ArCH_2O-$), 4/92 (1H, *m*, exchangeable, CH_2OH), 3·35 (1H, d, J = 3 Hz, H-4), 3·21 (1H, d, J = 3 Hz, sharpened by irr at 5·48 τ, H-6), two exchangeable 1H m at 1·61 and 0·88 (ArOH): mass spectrum: M, 174·0075 ($C_7H_7ClO_3$ requires 174·0084), *m/e* (relative abundance): 176(9), 174(27), 159(5), 158(33), 157(14), 156(100), 130(17), 128(53), 110(14), 103(5), 101(5), 100(16), 99(16), 65(15).

Acetylation with Ac_2O/py afforded in 74% yield the triacetyl derivative **12**, as an oil, R_f 0·52 ($CHCl_3$), ν_{max} (liquid film) cm^{-1}: 1770, 1745, 1230, λ_{max} (EtOH): no absorption >210 nm, NMR spectrum as in Table 1. Attempted PLC on Kieselgel HF_{254} using multiple elution resulted in hydrolysis to the oily diacetate **13**, R_f 0·35 ($CHCl_3$), ν_{max} (thin film) 3500 br, 1750, 1230, λ_{max} (EtOH) 315 nm (ε 2900): λ_{max} (EtOH-NaOH aq) 348 nm (ε 3400), NMR spectrum as in Table 1.

Diacetyl-α-resorcylic acid (**4**). Acetylation of α-resorcylic acid with Ac_2O/NaOAc gave the diacetate in 79% yield, as needles, m.p. 162° (lit.[4] m.p. 161-162°), ν_{max} (KBr): 3300-2800, 1770, 1695, 1593, 1225. NMR spectrum as in Table 1.

3,5-*Diacetoxybenzyl alcohol* (**5**). Diacetyl-α-resorcylic acid (1·49 g) in dry diglyme (20 ml) was treated at 0° under N_2 with a solution of B_2H_6 in dry THF (0·5M, 8 ml) and allowed to stand 30 min. After addition of water (150 ml), extraction with $CHCl_3$ and washing of the extract with $NaHCO_3$ aq and then water, evaporation gave the *diacetate* (**5**) as an oil (0·92 g, 66%), b.p. 105°/0·005 mm, R_f 0·80 (3% MeOH in $CHCl_3$), ν_{max} (thin film) cm^{-1}: 3520, 1760, 1210, λ_{max} (EtOH): no significant absorption >210 nm. NMR spectrum as in Table 1. (Found: C, 58·9; H, 5·5. $C_{11}H_{12}O_5$ requires C, 58·9; H, 5·4%).

3,5-*Diacetoxybenzyl acetate* (**6**). Acetylation of the alcohol **5** with Ac_2O/py gave after preparative TLC the triacetate **6** as an oil, b.p. 105°/0·01 mm, R_f 0·20 ($CHCl_3$), ν_{max} (thin film) cm^{-1}: 1770, 1740, 1619, 1595, 1200 br., λ_{max} (EtOH) 217 nm (ε 5800), 265 (300). NMR spectrum as in Table 1. (Found: C, 58·6; H, 5·2. $C_{13}H_{14}O_6$ requires: C, 58·7; H, 5·3%).

2-*Chloro*-3,5-*diacetoxybenzyl acetate* (**7**). 3,5-Diacetoxybenzyl acetate (176 mg) in HOAc (1 ml) was treated with a saturated solution of dry Cl_2 in HOAc (1 ml, *ca* 2 equiv) and the solution left at room temp for 24 hr. Preparative TLC of the oil left on evaporation, gave the *chloro derivative* (**7**) (120 mg, 60%) as an oil b.p. 115°/0·02 mm, R_f 0·28 ($CHCl_3$), ν_{max} (thin film) cm^{-1}: 1770, 1738, λ_{max} (EtOH) 227 nm (ε 6500), 276 (700). NMR spectrum as in table. (Found: C, 52·1; H, 4·5. $C_{13}H_{13}ClO$ requires: C, 51·9; H, 4·4%).

2-*Chloro*-3,5-*dihydroxybenzyl alcohol* (**2**). The above chloro compound (30 mg) in MeOH (10 ml) containing conc HCl aq (0·05 ml) was refluxed under N_2 for 6 hr. Evaporation gave an oily solid which afforded, upon crystallization from EtOAc-light petroleum, the *chlororesorcinol* 2 as needles (15 mg, 86%), m.p. 147°, R_f 0·24 (4% MeOH in $CHCl_3$), ν_{max} (KBr) cm^{-1}: 3370, λ_{max} (EtOH), 283 (ε 2370): λ_{max} (EtOH-NaOH) 303 (3840). NMR spectrum as in Table, mass spectrum *m/e* (relative abundance) 176 (33), 174 (100), 159 (5), 158 (7), 157 (18), 156 (18), 155 (11), 147 (9), 145 (28), 139 (73), 137 (18). [Found: C, 48·3; H, 4·3. M^+ at *m/e* 174 (Cl = 35). $C_7H_7ClO_3$ requires: C, 48·2; H, 4·0. M.W. 174·5].

Diacetylgentisic acid (**8**). Acetylation of the sodium salt of gentisic acid (0·5 g) with Ac_2O-HOAc (30 ml) in the presence of NaOAc (0·1 g) gave diacetylgentisic acid as rods (0·38 g, 56%), m.p. 120° (lit.[7] m.p. 118-119°), ν_{max} (KBr) cm^{-1}: 3500-2800, 1759, 1727, 1705, 1488, 1250, 1205, 850, λ_{max} (EtOH) 273 nm (ε 1670): λ_{max} (EtOH-NaOH aq) 294 nm (ε 2900). NMR spectrum as in Table. (Found: C, 55·7; H, 4·3. $C_{11}H_{10}O_6$ requires: C, 55·5; H, 4·2%).

5-*Acetoxysalicylic acid* (**9**). Diacetylgentisic acid (0·7 g) in aqueous 25% dioxan (80 ml) acidified to pH 6 with 6N HCl aq was refluxed for 2 hr. Extraction with $CHCl_3$ gave 5-*acetoxysalicylic acid* as needles (0·4 g, 67%), m.p. 130° (ether-light petroleum), ν_{max} (KBr) cm^{-1}: 1768, 1678, 1625, 1488, 1215, 838, 832, 804, 795, λ_{max} (EtOH) 308 nm (ε 3850): λ_{max} (EtOH-NaOH aq) 291 nm (ε 3920), 340 (2290). NMR spectrum as in Table (Found: C, 55·0; H, 4·1. $C_9H_8O_5$ requires: C, 55·1; H, 4·1%).

Methyl 5-*acetoxysalicylate* (**10**). Methylation of the above acid (50 mg) with ethereal CH_2N_2 at 0° for 1 min gave the corresponding *methyl ester* (**10**) as needles (45 mg, 84%), m.p. 87° (MeOH aq), R_f 0·51 ($CHCl_3$), ν_{max} (KBr) cm^{-1}: 1754, 1670, 1612, 1480, 873, 834, 782, λ_{max} (EtOH) 315 nm (ε 2960): λ_{max} (EtOH-NaOH aq) 344 nm (ε 3100). NMR spectrum as in Table. (Found: C, 57·0; H, 4·6. $C_{10}H_{10}O_5$ requires: C, 57·1; H, 4·8%).

Methyl 3-chloro-5-acetoxysalicylate (**11**). Methyl 5-acetoxysalicylate (0·15 g) in HOAc (1 ml) was treated with a saturated solution of Cl_2 in HOAc (3 ml) for 1 hr at room temp; addition of water (10 ml) afforded the *monochloro derivative* (**11**), which crystallized from MeOH aq in needles (0·15 g, 84%), R_f 0·43 ($CHCl_3$), ν_{max} (KBr) cm^{-1}: 1761, 1682, 1614, 1248, 1220, 802, 790, λ_{max} (EtOH) 311 nm (ε 3010); λ_{max} (EtOH–NaOH aq) 346 nm (ε 3430). NMR spectrum as in Table. (Found: C, 49·3; H, 3·7. $C_{10}H_9ClO_5$ requires: C, 49·1; H, 3·7%).

An exothermic reaction was found to occur when the chlorination was carried out by bubbling Cl_2 through a solution of **11** (0·35 g) in MeOH (15 ml) for 10 min. Evaporation and fractional crystallization of the residue afforded *carbomethoxytrichlorobenzoquinone* as yellow needles (0·18 g, 40%), m.p. 119°, R_f 0·46 ($CHCl_3$), ν_{max} (KBr) cm^{-1}: 1735, 1690, 1680, 1622, 865, 805, 776, 718, 690, λ_{max} (EtOH) 200 nm (ε 3070), 272 (3540) unchanged in base, NMR spectrum: τ 6·03 (3H, s, –OMe), mass spectrum: *m/e* (relative abundance) 274 (3), 272 (12), 270 (27), 286 (31), 244 (3), 243 (8), 242 (10), 241 (40), 240 (27), 239 (95), 238 (36), 237 (100), 215 (2), 213 (12), 212 (7), 211 (23), 210 (10), 209 (25). (Found: C, 36·1; H, 1·8. M^+ 268 (Cl = 35) $C_8H_3Cl_3O_4$ requires: C, 35·7; H, 1·1%; MW 269·5).

Synthetic 3-chlorogentisyl alcohol (**1**). Methyl 3-chloro-5-acetylgentisate (50 mg) in ether (10 ml) was added dropwise to a stirred suspension of LAH (0·15 g) in dry ether (10 ml) and the solution stirred at room temp for 1½ hr. EtOAc (20 ml) was carefully added and the ether allowed to evaporate. The complex was decomposed by addition of 5N HCl aq (5 ml) and stirring overnight. The EtOAc layer now afforded *3-chlorogentisyl alcohol* (**1**), which, after purification by prep TLC using EtOAc as eluant and crystallization from EtOAc–light petroleum, was obtained as needles (24 mg, 68%) m.p. 144°. (Found: C, 48·0; H, 3·9. C_7H_7ClO requires: C, 48·15; H, 4·0%), identical (R_f, m.m.p., IR spectrum) to a sample isolated from *P. canadense*.

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