Phytochemistry, Vol. 21, No. 12, pp. 2921–2923, 1982.
Printed in Great Britain.

0031-9422/82/122921-03$03.00/0

ASCOCORYNIN, A TERPHENYLQUINONE FROM *ASCOCORYNE SARCOIDES*

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(*Revised received* 29 *January* 1982)

Key Word Index—*Ascocoryne sarcoides*; Ascomycetes; terphenylquinones; antibiotics; ascocorynin.

Abstract—Ascocorynin, 2,5-dihydroxy-3-(*p*-hydroxyphenyl)-6-phenyl-1,4-benzoquinone, has been isolated from the fungus *Ascocoryne sarcoides*. The biogenetic implications arising from the formation of this compound are discussed.

INTRODUCTION

Terphenylquinones are widely distributed among the basidiomycetes [1, 2]. From mycelial cultures of *Ascocoryne sarcoides* (Jacquin ex S. F. Gray) Groves & Wilson (obtained from a spore print of its conidial state *Coryne dubia* Persoon ex S. F. Gray; syn. *Pirobasidium sarcoides* von Höhnel) we have isolated a new terphenylquinone (**1a**) which we have named ascocorynin. In the following paper, we wish to describe the fermentation, isolation, structural elucidation, and the antibiotic activity of **1a**, the structure of which is intermediate between polyporic acid (**2**) and atromentin (**3**). **1a** contains the same basic structural unit as peniophorin, whose X-ray analysis has recently been reported [3].

RESULTS AND DISCUSSION

Chemical investigations

Ascocorynin (**1a**), $C_{18}H_{12}O_5$ (high resolution MS), is obtained as green needles which are soluble in ethyl acetate, tetrahydrofuran and methanol, giving red solutions. In an alkaline solution ascocorynin turns dark violet, the colour of the fruiting bodies of *A. sarcoides*. The NMR spectrum shows two multiplets at δ 6.8 and 7.4 (intensity ratio 2:7), and the IR spectrum an intensive OH-band at 3300 cm^{-1}. Reaction with CH_2N_2 yields a trimethoxy compound (**1b**). The presence of a hydroxylated aromatic system may, therefore, be assumed. The lack of a carbonyl band at $\sim 1700\ \text{cm}^{-1}$ and the presence of two strong bands at 1610 and 1630 cm^{-1} suggest a quinone system. This is confirmed by the reduction of **1a** by $NaBH_4$ to a *leuco* compound and by the reductive acetylation [5] (Zn/Ac_2O) to a pentaacetate (**4b**). In agreement with structure **1a** is the MS fragmentation pattern which corresponds to that of **2** [6] and **3** [4], and the UV spectra of **1a** and **4b** which lie between those of **2**/**3** and **4a**/**4c**, respectively ($\lambda_{\max}$ nm: **2**, 261 and 332; **1a**, 262 and 353; **3**, 268 and 385; **4a**, 245; **4b**, 245; **4c**, 249 [7]). The structure **1a** is finally established by the following observations: (a) The NMR spectrum of **4b** shows three acetate signals (6:6:3H) at δ 1.92, 1.95, and 2.30, but in a *m*-terphenyl one acetate signal should be at δ 1.5 [8]. (b) If the aryl rings were adjacent, reaction with *o*-phenylenediamine [5] would give a mixture of phenazines. However, only a single product is obtained (NMR; TLC in two systems, viz. MeOH–H_2O–HOAc, 90:10:1, and BuOH–H_2O–HOAc, 10:2:1). (c) The NMR spectrum of **4b** shows in the aromatic region an AA′XX′ system (4 H) one half of which is superimposed by a symmetrical 5 H multiplet centred at δ 7.4. The shifts (δ 7.15 and 7.35) and splitting (8.5 Hz) of the visible part of the AA′XX′ system agree well with the data obtained [9] from **4c** (δ 7.13 and 7.33, 8.8 Hz). Upon addition of shift reagents these groups of signals (4 H-AA′XX′ system; 2 multiplets; 3 and 2 H) can be separated. All this evidence is in agreement only with the structure of 2,5-dihydroxy-3-(*p*-hydroxyphenyl)-6-phenyl-1,4-benzoquinone (**1a**).

1a has been obtained by Cain [10, 11] by reaction of 2,5-dichloro-1,4-benzoquinone with diazotized aniline and subsequently with diazotized 4-hydroxyaniline followed by hydrolysis. We could not repeat this synthesis since we obtained in the first coupling step 2,5-dichloro-3,6-diphenyl-1,4-benzoquinone. Spectral data have not been given by Cain.

The occurrence of **1a** sheds some light on the biosynthesis of terphenylquinones. For the formation of **1a** two pathways may be considered i.e. (a) enzymatic hydroxylation of **2** or (b) condensation of phenylpyruvic acid (**6**) with *p*-hydroxyphenylpyruvic acid (**7**) (see Scheme 1).

Read *et al.* [12] explain the biosynthesis of **3** by condensation of two molecules of **7**; Herrmann [9] discusses enzymatic hydroxylation of **2** via **1a** to **3**, although feeding studies with DL-[3′ – ^{13}C]tyrosine and DL-phenyl[3 – ^{13}C]alanine showed that in the fruiting bodies of *Paxillus atrotomentosus* **3** is formed directly from **7** without the intermediacy of **2**. In

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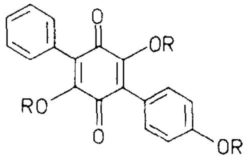
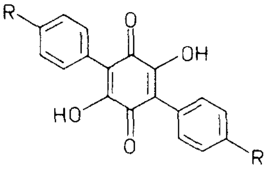
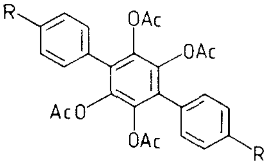


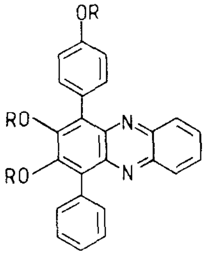
1a R=-H
1b R=Me



2 R=-H
3 R=-OH



4a R=-H, R'=-H
4b R=-H, R'=-OAc
4c R=-OAc, R'=-OAc



5a R=-H
5b R=Me

A. sarcoides neither **2** nor **3** were detected, therefore, the biogenetic pathway (b) seems to be more likely. A final decision would obviously call for feeding studies with labelled precursors.

Biological activity

In the serial dilution test growth of several Gram-positive bacteria was inhibited by ascocorynin at moderate concentrations, whereas Gram-negative bacteria are not affected at concentrations up to 100 μg (Table 1).

In the plate diffusion test 100 μg ascocorynin per paper disc did not interfere with the growth of the fungi *Hansenula anomala, Saccharomyces cerevisiae, Aspergillus panamensis, Penicillium chrysogenum, Botrytis cinerea* and *Rhodotorula glutinis.*

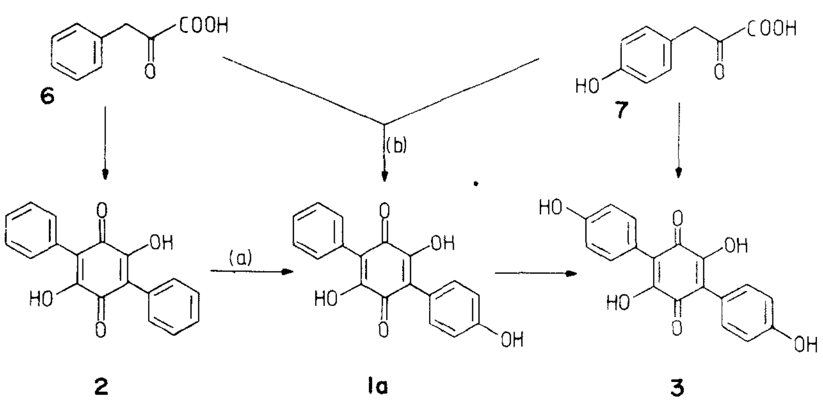
EXPERIMENTAL

General. MS: 70 eV, direct insertion; ^{1}H NMR: 60 or 90 MHz, TMS as int. standard; IR: KBr. Mps are uncorr. TLC: Merck 60 F_{254} Si plates and on polyamide; HPLC on 16 mm × 250 mm columns, which were packed with Polygosil 60-D 10, by the viscosity method [13].

Fermentation. The mycelial culture of *A. sarcoides* (strain WQ 6979) was obtained from a spore print of its conidial state. It was maintained on agar slants of YMG-medium (g/l.): yeast extract (4), malt extract (10), glucose (4). This mycelium was used to inoculate a 500-ml conical flask containing 130 ml of a modified Raulin's solution, which was composed of (g/l.): cane sugar (46.66), tartaric acid (2.66), NH_4NO_3 (2.66), K_2CO_3 (0.4), $(NH_4)_2HPO_4$ (0.4),

Table 1. Antibacterial spectrum of ascocorynin (**1a**)

Bacterium	MIC (μg/ml)
Arthrobacter citreus	70–80
Bacillus brevis	70–80
Bacillus stearothermophilus	20–30
Bacillus subtilis	> 100
Corynebacterium insidiosum	50–60
Escherichia coli	> 100
Micrococcus roseus	40–50
Mycobacterium phlei	70–80
Proteus vulgaris	> 100
Pseudomonas fluorescens	> 100
Sarcina lutea	40–50
Staphylococcus aureus	50–60
Streptomyces viridochromogenes	50–60
Xanthomonas oryzae	> 100



Scheme 1.

$4MgCO_3 \cdot Mg(OH)_2 \cdot 4H_2O$ (0.27), $(NH_4)_2SO_4$ (0.17), $ZnSO_4 \cdot 7H_2O$ (0.047), $FeSO_4 \cdot 7H_2O$ (0.047), adjusted with HCl to pH 5. 100 ml of this culture served as inoculum for a 5-l. conical flask, containing 1.5 l. of the modified Raulin's solution. This flask was incubated for 10 days on a rotary shaker (120 rpm) at room temp.

Isolation. The olive-coloured culture was harvested and the culture fluid separated from the mycelium. The fluid was adjusted to pH 2 and extracted with 750 ml EtOAc (×2). After evapn of the solvent crude ascocorynin was obtained. Several recrystns from tetrahydrofuran yielded 500 mg of the pure terphenylquinone. No other coloured products could be detected by TLC. (TLC properties of **1a**. Si gel 60: MeCOEt–H_2O–H·CO_2H (250:25:1), R_f 0.5; Et_2O-satd with HCl, R_f 0.8; *n*-BuOH–H_2O–HOAc (4:1:1), R_f 0.55. Polyamide: MeCOEt–H_2O–HCO_2H (250:25:1), R_f 0.18 [4]).

Ascocorynin (*2,5-dihydroxy-3-*(p-*hydroxyphenyl*)*-6-phenyl-1,4-benzoquinone*) (**1a**) Mp > 300° (sublim.); MS *m/z* (rel. int.): 310 (14),* 309 (21), 308.06857 (calcd. 308.06847 for $C_{18}H_{12}O_5$; M^+; 100), 290 (5), 280 (19), 263 (6), 262 (8), 234 (7), 223 (10), 207 (8), 205 (6), 189 (18), 145 (20), 134 (20), 89 (18); ^1H NMR (CD_3OD/CCl_4): δ 6.7 (*d*(*br*), 7.5 Hz, 2 H); 7.3 (*m*, 7 H); IR ν (strong bands underlined) cm^{-1}: $\underline{3300}$, $\underline{1630}$, $\underline{1610}$, 1515, 1445, $\underline{1320}$, $\underline{1250}$, 1190, $\underline{1000}$, 845, 805, 775, 720; UV λ (dioxan) nm (ϵ): 262 (39 100), 353 (5100), 470 (490).

1,2,4,5-Tetraacetoxy-3-(p-*acetoxyphenyl*)*-6-phenylbenzene* (**4b**). Upon addition of 10 drops of pyridine and a small amount of Zn powder to 31 mg (0.1 mmol) of **1a** in 2 ml Ac_2O the red soln was decolourized. After 15 min the reaction mixture was diluted with 50 ml EtOAc and after removal of the remaining Zn by filtration treated with 50 ml H_2O and extracted with 50 ml EtOAc (×3). Recrystn from EtOAc–hexane and purification by HPLC (hexane–*iso*-PrOH, 2:1) yielded 43 mg (83%) of **4b**. Mp 198–200°; MS 520.1364 (calcd 520.1369 for $C_{28}H_{24}O_{10}$; M^+; 1), 478, 1250 (calcd 478.1263 for $C_{26}H_{22}O_9$, $M - CH_2CO$, 7), 436 (18), 394 (35), 352 (100), 310 (33), 291 (13), 263 (3), 207 (2), 189 (1), 89 (2), 43 (30); ^1H NMR ($CDCl_3$): δ 1.92 (*s*, 6 H), 1.95 (*s*, 6 H), 2.3 (*s*, 3 H), 7.15 (*d*, 2 H, 8.5 Hz), 7.35 (*d*, 2 H, 8.5 Hz), 7.4 (*m*, 5 H); IR ν cm^{-1}: $\underline{1765}$, 1760, 1750, 1510, 1455, 1430, 1405, $\underline{1370}$, 1265, $\underline{1190}$, 1175, 1160, 1100, $\underline{1025}$, 980, 915, 875, 805. UV λ (dioxan) nm (ϵ): 245 (19 460).

1a suspended in CH_2Cl–MeOH (1:1) was treated with CH_2N_2. Purification by HPLC (hexane–*iso*-PrOH, 100:1) yielded 5% of a compound which was analysed by MS: *m/z* (rel. int.): 350 (82), 335 (7), 322 (20), 317 (18), 307 (22), 279 (24), 251 (79), 89 (100). The spectrum is consistent with structure **1b** the trimethyl ether of **1a**.

1-(p-*Hydroxyphenyl*)*-2,3-dihydroxy-4-phenylphenazine* (**5a**). To 31 mg (0.1 mmol) **1a** dissolved in 3 ml boiling HOAc 70 mg (0.65 mmol) of *o*-phenylenediamine in 1 ml HOAc were added under stirring. The mixture upon refluxing for 45 min turned dark red. The precipitated **5a** was filtered off and washed with 50 ml hexane. Yield 25 mg (66%). Mp > 300° (sublim.); MS *m/z* (rel. int.): 382 (9),* 381 (28), 380.1150 (calcd 380.1161 for $C_{24}H_{16}N_2O_3$; M^+: 100), 379 (53), 364 (40), 363 (49), 362 (27), 361 (39), 345 (23), 334 (16), 210 (17), 201 (20), 153 (39); ^1H NMR (DMSO): δ 6.9 (*d*, 2 H, 8 Hz), 7.3 (*d*, 2 H, 8 Hz), 745 (*m*, 6 H), 7.8 (*m*, 2 H); IR cm^{-1}: $\underline{3280}$, $\underline{1615}$, $\underline{1555}$, $\underline{1540}$, 1490, $\underline{1390}$, $\underline{1370}$, 1330, $\underline{1275}$, $\underline{1250}$, 1040, $\underline{1020}$, 780, 700: UV λ (dioxan) nm (ϵ): 266 (25 740), 383 (7410), 408 (sh), 505 (2160).

1-(p-*Methoxyphenyl*)*-2,3-dimethoxy-4-phenylphenazine* (**5b**). 10 mg (0.026 mmol) of **5a** were suspended in 20 ml MeOH–CH_2Cl_2 (1:1) and treated with CH_2N_2. Purification by HPLC (Polygosil 60-D10, hexane–*iso*-PrOH, 20:1) yielded 9.8 mg (88%) **5b**. MS *m/z* (rel. int.): 422.1632 (calcd 422.1630 for $C_{27}H_{22}N_2O_3$, M^+, 74), 421 (32), 407 (15), 392 (37), 391 (100), 376 (24), 375 (21), 361 (25), 360 (28), 195.5 (25), 188 (24), 152 (20), 146 (23); ^1H NMR (Me_2CO-d_6, 60 MHz): 3.76 (*s*, 3 H), 3.78 (*s*, 3 H), 3.92 (*s*, 3 H); IR ν cm^{-1}: 1605, 1505, $\underline{1385}$, $\underline{1305}$, 1290, $\underline{1250}$, 1175, 1135, 1115, $\underline{1055}$, 990, 760; UV λ (dioxan) nm (ϵ): 263 (55 200), 373 (12 800), 415 (sh).

Test organisms and antibacterial spectrum. All bacteria were grown on Nutrient Broth (Difco). Size of inoculum: 2×10^6 cells/ml. The incubation temp. was 27° for *Micrococcus roseus*, *Corynebacterium insidiosum*, *Sarcina lutea*, *Pseudomonas fluorescens* and *Arthrobacter citreus*; 55° for *Bacillus stearothermophilus*, and 37° for the other bacteria. The yeasts were grown on Yeast Nitrogen Base (Difco), containing 0.4% glucose, and cells or conidia of the filamentous fungi were seeded in a malt extract medium (20 g malt extract, 15 g agar/l.). All fungi were incubated at 27°.

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*Quinones [14] and phenazines (see **5a**) [15] frequently give $[M+2]^+$ ions due to partial hydrogenation in the ion source. The otherwise unexpected *m/z* 364 from **5a** could thus be $[M+2-H_2O]^+$.