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ASPERPENTYN, A NOVEL ACETYLENIC CYCLOHEXENE EPOXIDE FROM *ASPERGILLUS DURICAULIS**

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Key Word Index—*Aspergillus duricaulis*; fungi; asperpentyn; 3-(3-methyl-but-3-en-1-ynyl)-7-oxabicyclo[4.1.0]hept-3-ene-2,5-diol.

Abstract—Reinvestigation of antimicrobial extracts from *Aspergillus duricaulis* yielded the novel acetylenic compound asperpentyn.

INTRODUCTION

During the course of a screening programme for novel substances with antimicrobial activity, we reported the isolation of several new aromatic metabolites from the culture broth of the fungus *Aspergillus duricaulis*. Structurally, these compounds are phthalides, furochromanols and phthalimidines [1, 2]. A reinvestigation of fermentation extracts from this strain using improved separation methods have now led to the isolation of asperpentyn (**1**), a novel compound with an unique cyclohexene epoxide structure.

RESULTS AND DISCUSSION

Asperpentyn (**1**) was detected as a minor metabolite in the course of the purification of the furochromanols [1], which constitute the main components of the extract. Compound **1** is not stable in contact with silica gel. Therefore, the crude extract was subjected to partition chromatography using water-loaded Sephadex G-10 and petrol–ethyl acetate as eluent. The fraction of furochromanols separated by this method was crystallized, and from the mother liquors **1** was isolated by multiple stage chromatography on organic materials exclusively.

The molecular composition of asperpentyn (**1**) was established as $C_{11}H_{12}O_3$ by high resolution mass spectrometry of the $[M]^+$. From UV and IR spectra the structural element of a hepta-2,6-dien-4-yn-1-ol was deduced: extinction maxima at 258 and 268 nm are in agreement with an expected 30 nm bathochromic shift on the values reported for non-2-en-4-yn-1-ol [3]. The ^{13}C NMR spectra show the signals of one methyl group, four sp^3 methine carbons attached to oxygen atoms, four olefinic carbons (two singlets, one doublet, one triplet), and in addition two singlets of low intensity at δ 89.3 and 91.0, which account for the triple bond in **1**. This acetylenic group is corroborated by the IR band at 2100 cm^{-1}.

The ^1H NMR spectrum and $^1H\{^1H\}$-decoupling experiments establish the partial structures **a** and **b** ($C_6D_6 + D_2O$; δ ppm, J = Hz). These structural elements together with the acetylenic group and two readily exchangeable hydrogens (mass spectrum, ^1H NMR) represent all the atoms present in asperpentyn and indicate the cyclic structure **1**.

The relative configuration shown in **1** was deduced from the ^1H NMR coupling constants with the aid of

*Part 36 in the series 'Metabolites of Microorganisms'. For part 35 see ref. [11].

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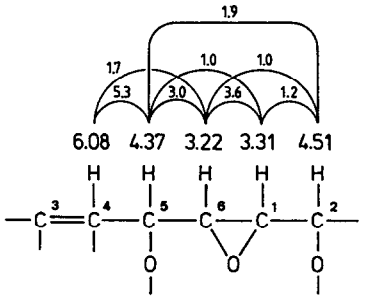

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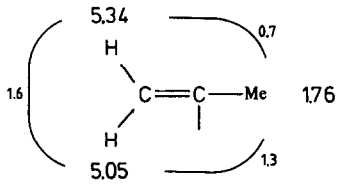

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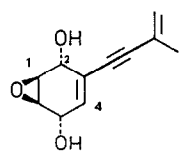

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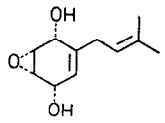

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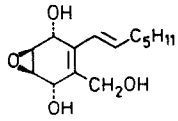

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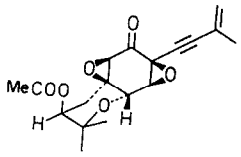

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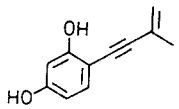

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Dreiding models: the coupling constants† of the ABC spin system H-4, H-5, and H-6 (see **a** and **b**) demand dihedral angles in the range 20–60° or 110–130° between H-4 and H-5 (3.6–6.3 Hz) [4] and 40–60° or 120–140° between H-5 and H-6 (1.7–4.3 Hz) [5]. Therefore, the OH group at C-5 must be pseudo-axial in the 'closed boat' conformation of the 7-oxabicyclo[4.1.0]hept-3-ene ring system [6]. At the other side of the epoxy group, an allylic coupling between the protons H-2 and H-4 was not detectable at a spectral resolution of 0.2 Hz. Therefore, the angle between H-2 and the substituent at C-3 has to be in the range 20–50° [4]; thus, the hydroxyl group at C-2 also is in a pseudo-axial position. Similar considerations on related molecules have been reported by Prinzbach [6], H. Fritz, (personal communication), and Rickards [7]. The spectroscopic data reported [6, 7] support our conclusions.

Asperpentyn belongs to the comparatively rare class of highly oxygenated natural cyclohexanoid compounds, which occur in microorganisms as well as in plants. They exhibit biological activities ranging from antifungal, antibacterial and antitumour to phytotoxic and enzyme inhibitory. To our knowledge, the only natural cyclohexanoids with a substitution pattern similar to **1** (namely 7-oxabicyclo[4.1.0]hept-3-ene-2,5-diols) are 7-desoxypanepoxydol (**2**) (from *Panus*, Basidiomycetes) [8] and the antifungal eupenoxide (**3**) (from *Eupenicillium*) [7]. However, by its acetylenic side chain, **1** represents a unique structural feature in this group. From this point of view, it resembles the antibiotic oxirapentyn (**4**) (from *Beauveria felina*, Deuteromycetes) [9] and the phenol **5** (*Helminthosporium siccans*, Deuteromycetes) [10]. Asperpentyn, however, is not active against *Botrytis cinerea* and *Bacillus subtilis*.

†In contrast to substituted cyclohexene epoxides of higher symmetry [6,7, H. Fritz (personal communication)], the ^{1}H NMR spectra of asperpentyn could be interpreted by a first order analysis.

EXPERIMENTAL

^{1}H NMR were recorded at 90 MHz, ^{13}C NMR at 22.5 MHz, int. ref. TMS; chemical shift values reported in δ (ppm). MS were obtained by direct inlet at 70 eV.

Material. Fermentation of *Aspergillus duricaulis* Raper & Fennell (CBS 481.65; Tü 679) and extraction of the culture broth were carried out as described in [11].

Isolation. Crude ext (5 g) was subjected to the following sepn procedures: (i) partition chromatography on 150 g Sephadex G-10/H_2O (stationary phase) with petrol–EtOAc (3:2); (ii) concn in the EtOAc mother liquor of the main compounds; (iii) cf. (i); (iv) consecutive chromatography on 15g Fractogel PVA-500 (Merck) with (1) EtOAc; (2) cyclohexane–EtOAc (1:1) and (3) EtOAc. Yield: 5.7 mg asperpentyn (**1**).

(−)-(1R*, 2S*, 5R*, 6S*)-3-(3-*methylbut*-3-*en*-1-ynyl)-7-*oxabicyclo*[4.1.0]*hept*-3-ene-2,5-*diol*; *asperpentyn* (**1**). Colourless oil. R_f 0.15 on silica gel, $CHCl_3$–MeOH (49:1); anisaldehyde reagent [12], heating at 110° for 10 min: deep blue colour. $[\alpha]_D^{20}$ −20° (Me_2CO; *c* 0.1). UV λ_{max}^{MeOH} nm (log ε): 214 infl. (3.7), 240 infl. (3.7), 257 (3.8), 267 infl. (3.8). CD: $\Delta\varepsilon_{210}$ +0.3, $\Delta\varepsilon_{260}$ −0.3 (MeOH; *c* 0.001). IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3400, 2930, 2860, 2100 (w), 1020, 905. ^{1}H NMR ($C_6D_6 + D_2O$): see **a** and **b**. ^{1}H NMR (acetone-d_6 + D_2O): δ5.90 (1H, *dd*, $J_{4,5}$ = 5.1 Hz, $J_{4,6}$ = 1.6 Hz, H-4), 5.23 (2H, *m*, C=CH_2), 4.35 (1H, *m*, $J_{5,4}$ = 5.1 Hz, H-5), 4.24 (1H, *m*, H-2), 3.24 (2H, *m*, H-1 and H-6), 1.82 (3H, *dd*, *J* = 1.6 Hz, *J* = 1.2 Hz, Me). ^{13}C NMR (acetone-d_6): δ133.0 (*d*, C-4), 127.8 (*s*, C-3), 123.0 (*s*, C-3'), 122.3 (*t*, C-4'), 91.0 and 89.3 (2*s*, C-1' and C-2'), 65.8 and 63.3 (2*d*, C-2 and C-5), 54.1 and 52.9 (2*d*, C-1 and C-6), 23.4 (*q*, C-5'). EIMS *m/z* (rel. int.): 192 $[M]^+$ (22), 145 (30), 117 (20), 115 (24), 93 (27), 91 (100), 79 (51), 77 (69), 71 (99), 69 (32), 65 (42), 63 (27), 57 (23), 55 (49), 53 (28), 51 (46), 50 (23), 43 (73), 41 (63); $[M]^+$ after treatment with MeOD: 194 (1), 193 (5), 192 (8). HRMS: *m/z* 192.07858 ($C_{11}H_{12}O_3$ requires 192.07864), 91.05475 (C_7H_7 requires 91.05477), 71.01333 ($C_3H_3O_2$ requires 71.01330).

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