EV-22, a Novel Antifungal Triacetylenic Dioxolone

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The structure of EV-22, an antifungal antibiotic isolated from the fermentation broth of a *Microbispora sp.*, has been assigned an unusual triacetylenic dioxolone structure based upon chemical transformations and spectroscopic data.

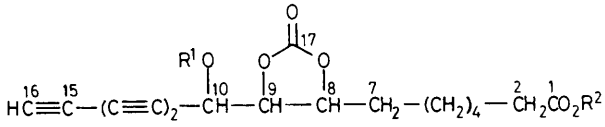
As part of a continuing search for antifungal agents produced by micro-organisms, we report the isolation and characterization of a novel triacetylenic dioxolone, EV-22, produced by *Microbispora sp.*[1] During the isolation it was found that the antifungal activity was destroyed on concentration of the organic extracts. The final purified material was found to be unstable except in the form of dilute solutions. Many polyacetylenic antifungals, bearing a partial structural relationship to EV-22, have been reported.[2]

Structure (**1**) was assigned to the major component of the antifungal extracts based upon a combination of chemical modification, degradations, and physicochemical methods. Thus methanolysis of (**1a**) in methanolic hydrochloric acid gave the methyl ester (**1b**) in high yield and subsequent acetylation gave the *O*-acetate (**1c**). All three compounds were too unstable to concentrate sufficiently to obtain comprehensive spectroscopic data, however, the ^{1}H and ^{13}C n.m.r., i.r., and mass spectroscopic evidence for (**1b**) and (**1c**) led to a partial assignment of the structure.†

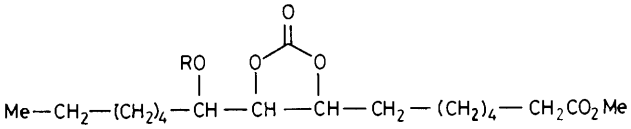
For (**1b**), i.r. absorptions (cm^{-1}) were at 1810, 1755, 1725 (s), and 2250 (vw). Evidence for the presence of a dioxolone moiety, highly unusual in a natural product, was obtained from the i.r. spectrum ($\nu_{max.}$ 1810 cm^{-1}) and from the ^{13}C spectrum (δ 153.1 assigned to C-17).[3]

The presence of the triacetylenic chromophore in (**1b**) was established both by the distinctive u.v. chromophore ($\lambda_{max.}$ 255, 270, 287, 305 nm) and by the observation that, upon hydrogenation twelve hydrogens were taken up by (**1b**) to give the fully saturated compound (**2a**) as reflected in its mass spectrum (M_r 344).

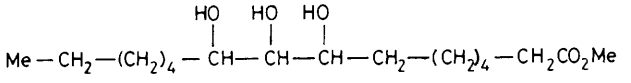
From the ^{1}H n.m.r. data, the OH function was assigned to C-10 of (**1b**). Upon acetylation, the carbinol proton of (**1b**) (δ 4.3—4.7) shifted downfield to δ 5.61 (*J* 5.5 Hz) in (**1c**). The decoupling experiments confirmed that the doublet at δ 5.61 in the spectrum of (**1c**) was coupled to a triplet (*J* 5.5, 5.5 Hz) at δ 4.40. This triplet was subsequently assigned to H-9. The presence of a methine proton resonance at δ 4.56 (dt, *J* 5.5, 6.5 Hz), assigned to H-8, defined the contiguous nature of the three carbons bonded directly to oxygen. The calculated dihedral angle[4] between H-8 and H-9 is approximately 128°, consistent with a β,α relationship with a dihedral angle of *ca.* 130° between the protons. A comparable relationship also exists between H-9 and H-10. Similar *J* values [H(8,9) *J* 5.5 Hz and H(9,10) *J* 5.5 Hz] were also found in (**2a**) and (**4a**), except that *J* H(9,10) was slightly smaller in (**2a**), indicative of greater carbon chain flexibility upon reduction. The ^{13}C n.m.r. spectrum of (**1c**) indicated the presence of six methylene, four methine, one OCH_3, one $COCH_3$ and three C=O carbons. In addition, a single frequency off resonance (SFOR) experiment confirmed that the δ 5.61 proton resonance was related to the δ 63.5 C-10. The methine carbon absorption at δ 68.9 in the ^{13}C spectrum of (**1c**) can be assigned to the terminal acetylenic carbon. The presence of an attached hydrogen presumably accounts for the fact that this was the only acetylenic carbon observed in the spectrum. Under the conditions of the experiment the others were not observed as a consequence, presumably, of the long T_1 of non-protonated acetylenic carbon. In accord with this assignment, there is no corresponding resonance in the spectrum of the hydrogenated



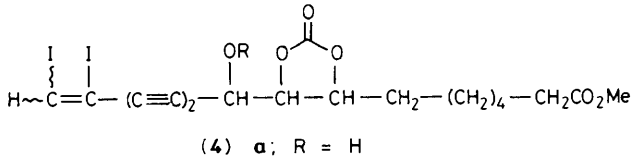
(**1**) **a**; $R^1 = R^2 = H$
b; $R^1 = H$, $R^2 = Me$
c; $R^1 = Ac$, $R^2 = Me$



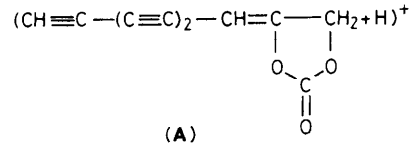
(**2**) **a**; R = H
b; R = Ac



(**3**)



(**4**) **a**; R = H
b; R = Ac



(**A**)

† ^{1}H n.m.r. (δ, $CDCl_3$): (**1b**), 1.35—1.60 (CH_2), 2.30 (CH_2CO), 3.65 (OCH_3), 2.20 (≡CH), and 4.3—4.7 (CHO–); (**1c**), 1.20—1.80 (CH_2), 2.35 (t, *J* 7.5 Hz, CH_2CO), 3.70 (OCH_3), 2.23 (≡CH). 2.18 ($COCH_3$), 4.40 (dd, *J* 5.5, 5.5 Hz, CHO), 4.65 (dt, *J* 5.5, 6.5 Hz, CHO–), and 5.60 (d, *J* 5.5 Hz, CHO–). ^{13}C n.m.r. (δ, $CDCl_3$): (**1b**), 24.7, 25.5, 29.5, 29.4, 34.5, 34.5 (C-2 to C-7), 51.9 (OCH_3), 63.0, 71.0, 79.3, 82.3 (C-8, 9, 10, 16), 155.1 (C-17), and 174.8 (C-1); (**1c**), 24.0, 24.7, 28.7, 28.8, 33.9, 34.4 (C-2 to C-7), 51.6 (OCH_3), 63.5, 68.9, 79.2, 78.3 (C-8, 9, 10, 16), 153.1 (C-17), 174.0 (C-1), 168.7 ($COCH_3$), and 20.5 ($COCH_3$).

Mass spectra [chemical ionisation (c.i.) isobutane]: (**1b**), no molecular ion was observed. A weak ion at *m/z* 399 (4%) was possibly due to some contaminant. The other fragments are at *m/z* 73, 141, 145, 173 (100%), 183, 197, 215, 227, 229, 259. The most intense ion at *m/z* 173 corresponded to (**A**).

derivative (**2a**). Futher evidence for the terminal position of the triacetylenic group was provided by the presence of an absorption at δ 2.23 (≡CH) in the ^{1}H n.m.r. spectrum of (**1c**). This absorption is absent in the spectrum of (**2a**), which does, however, contain an absorption at δ 0.92 corresponding to a terminal methyl group (δ ^{13}C, 14.0).‡

Decoupling experiments with (**2a**) confirmed the assignments of resonances at δ 2.12 (dt, H-11), 2.34 (t, H-2), 3.62 (m, H-10), 4.16 (dd, H-9), and 4.63 (dt, H-8). Chemical ionisation mass spectroscopy (c.i.m.s.) showed *m/z* 345 $(M + 1)^+$ and 362 $(M + NH_4)^+$. Upon acetylation (**2b**) was obtained in which H-10 was shifted downfield to δ 4.28 and c.i.m.s. gave *m/z* 387 $(M + 1)^+$ and 404 $(M + NH_4)^+$ consistent with the structure. During the formation of (**2a**) and its subsequent conversion into (**2b**), partially reduced products [(**2c**), *m/z* 343 $(M + 1)^+$ and 360 $(M + NH_4)^+$ and its acetate (**2d**) (*m/z* 384)] were found, having a 13,14 double bond on the basis of ^{1}H, ^{13}C n.m.r., and mass spectral data. Partially reduced (**2c**), verified by high resolution electron impact mass spectroscopy (h.r.e.i.m.s.) ($C_{18}H_{30}O_6$ 342.2036 (*M*), $C_{11}H_{18}O_5$ 230.1139, and $C_{11}H_{15}O_5$ 227.0912), could be further converted into (**2b**).

Treatment of (**2a**) with methanolic sodium hydroxide gave the triol (**3a**) having the appropriate molecular ion in the mass spectrum (*m/z* 318), confirmed by the formation of triacetate (*m/z* 444) and trisilyl (*m/z* 534) derivatives. In addition, the reaction mixture also contained partially reduced triol with one double bond, (**3b**) (*m/z* 316), confirmed by the formation of a trisilyl derivative (*m/z* 532).

Addition of iodine to solutions of (**1b**) and (**1c**) produced intensely u.v.-active and stable products as mixtures of *cis* and *trans* isomers of (**4a**) and (**4b**), respectively. Addition took place at the terminal group (C-15,16) as shown by the absence of an absorption at δ 68.9 and the appearance of a new resonance at δ 92.6 (assigned to C-16) in the ^{13}C n.m.r. spectrum of (**4a**).§ Structure (**4a**) was verfied by h.r.e.i.m.s. ($C_{18}H_{20}O_6I$ 459.0279 (*M* − I), $C_{11}H_{15}O_5$ 227.0945, and $C_8H_{15}O_2$ 143.1029).

Compounds (**1a**), (**1b**), and (**4a**) displayed activity *in vitro* against a range of *Candida albicans* and dermatophytes. However, no activity was observed *in vitro* in *C. albicans* hamster vaginal model. The *in vitro* activity was greatly reduced in the presence of serum suggesting very strong protein binding or inactivation by the serum. The di-iodo derivative (**4a**) may be a pro-drug after dissociation of iodine and its *in vitro* activity was comparable to that of (**1b**).

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‡ ^{1}H n.m.r. (δ, $CDCl_3$): (**2a**), 0.92 (t, *J* 7.0 Hz, CH_3), 1.34—1.60 (CH_2), 2.30 (CH_2CO), 3.70 (OCH_3), 4.16 (dd, *J* 6.0, 3.0 Hz), 4.63 (dt, *J* 6.0, 6.0, 5.0 Hz), 3.62 (br.), and 2.12 (d, *J* 7.0 Hz, OH). ^{13}C n.m.r. (δ, $CDCl_3$): (**2a**), 24.3, 24.7, 28.7, 29.0, 33.9, 34.0 (C-2 to 7), 51.5 (OCH_3), 70.9, 78.5, 83.5 (C-8 to 10), 154.6 (C-17), 174.1 (C-1); additional carbons 32.9, 31.6, 28.7, 25.4, 22.5 (C-11 to 15), and 14.0 (C-16).

§ ^{1}H n.m.r. (δ, $CDCl_3$): (**4a**), 1.35—1.65 (CH_2), 2.30 (CH_2CO), 3.70 (OCH_3), 4.28, 4.56, 4.70 (CHO–), and 7.58 (=CHI). ^{13}C n.m.r. (δ, $CDCl_3$): (**4a**), 24.0, 24.7, 28.6, 28.8, 34.0, 34.3 (C-2 to 7), 51.5 (OCH_3), 63.3 (C-10), 78.3, 81.4 (C-8,9), 153.9 (C-17), 174.4 (C-1), and 92.6 (C-16). E.i.m.s.: (**4a**), 571 (*M* −15); (**4b**), 628 (*M*).