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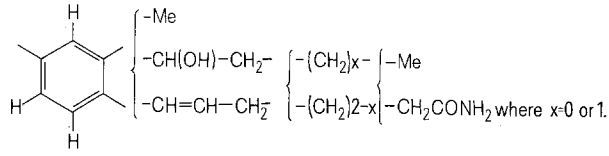
The Structure Determination of a Novel C_{17} Metabolite from Streptomyces X-537 using Eu(DPM)$_3$

During a study of the fermentation yields of antibiotic X-537A[1], we isolated crystals of (I), from the mother liquors of the broth ethyl acetate extract. From microanalysis and mass spectrometry, the molecular formula of (I), m.p. 119–121°, was determined as $C_{17}H_{25}NO_2$(M^+ 275) and UV- and IR-spectroscopies revealed λ_{max} (EtOH) 211 (26,100), 252 (15,800) nm (ε) and ν_{max} (KBr) 1650, 3200, 3380 cm^{-1}.

Acetylation of (I) with acetic anhydride in pyridine gave (II), $C_{19}H_{27}NO_3$, m.p. 84°, λ_{max} (EtOH) 210 (26,000), 241 (14,900) nm (ε); ν_{max} ($CHCl_3$) 1680, 1728, 3415, 3580 cm^{-1}. Jones oxidation of (I) gave the ketone (III), $C_{17}H_{23}NO_2$, m.p. 59–60°, M^+ 273, λ_{max} (EtOH) 234 (19,000), 260 (10,500), 307 (2000) nm (ε); ν_{max} ($CHCl_3$) 1680 cm^{-1}, and treatment of (I) with 5% sodium hydroxide in aqueous methanol (1:1 v/v) gave the acid (IV), $C_{17}H_{24}O_3$, m.p. 127-8°, ν_{max} ($CHCl_3$) 1710, 3510 cm^{-1}.

A closely related compound, *trans*-6-[*trans*-2-(1-butenyl)-4-tolyl]hex-5-enamide (V), $C_{17}H_{23}NO$, m.p. 127–128°, λ_{max} (EtOH) 239 nm (ε 27,800), 264 (21,200); ν_{max} (KBr) 960, 1665, 3420 cm^{-1}, has also been isolated from the X-537 fermentation. Furthermore, (V) can be readily obtained from (I) by dehydration using *p*-toluene sulphonic acid in refluxing benzene.

The structure of (I) was determined by proton NMR-spectroscopy with the aid of *tris*(dipivalomethanato)-europium-induced shifts[2]. The spectrum in DMSO-d_6 (Figure) exhibited peaks at δ 0.88 (t, 3, CH_3CH_2), 1.10–1.90 (m, 6, $3CH_2$), 2.00–2.40 (m, 4, $2CH_2$), 2.28 (s, 3, CH_3-Ar), 4.80 (m, 1, ArC*H*OH), 4.95 (d, 1, J 4 Hz, CHO*H*), 5.99 (d of t, 1, J *trans* 15 Hz and J vic 6.5 Hz, $-CH_2CH$=CH–), 6.62 (d, 1, J *trans* 15 Hz, –CH=C*H*-Ar), 6.65, 7.22 (br, 2, $-CONH_2$), and 7.26, 7.22, 6.94 (ABX, 3, J_{AX} ortho 8 Hz, J_{BX} meta 2 Hz, 1, 2, 4-trisubstituted benzene ring). From these data, the following partial structure could be deduced:



Using Eu(DPM)$_3$ in $CDCl_3$, it was possible to select a unique structure (I). In particular, the number of methylene groups in each chain was deduced; secondly, the chain-terminating groups of the carbinol and olefin aromatic substituents were shown to be $-CH_3$ and $-CONH_2$, respectively. These deductions were based on the following results. Addition of 10 mg of Eu(DPM)$_3$ to a solution containing 20 mg of (I) in $CDCl_3$ (0.3 ml) showed that the two *lowfield* CH_2 groups (δ 2.21 and 2.24) adjacent to $CONH_2$ and C=C were shifted paramagnetically 2.35 (triplet) and 1.10 ppm (quartet), respectively, whereas the C*H*OH proton was shifted only 0.50 ppm. The three *high-field* CH_2 groups (δ 1.38, 1.68, and 1.77) gave shifts of 0.21 (sextet), 0.33 (quartet), and 1.94

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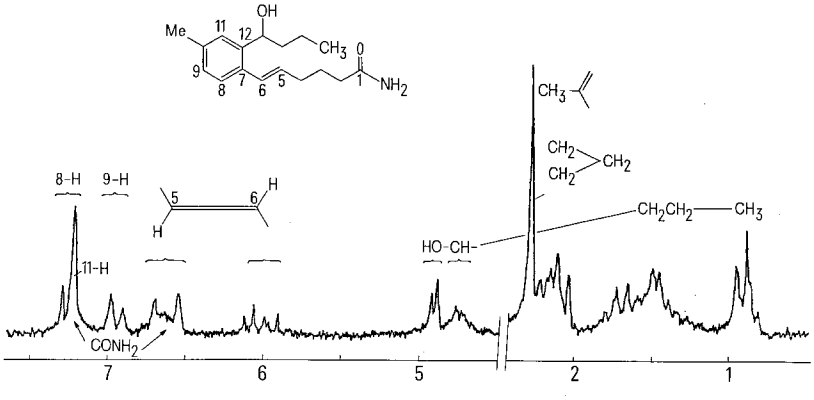


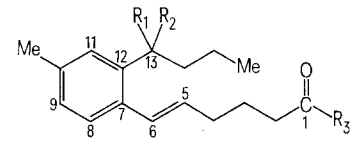
100 MHz proton NMR-spectrum of (I) in DMSO-d_6 solution.

(quintet), respectively, and the relative magnitudes of these shifts indicated that the quintet was due to a CH_2 group β to the amide carbonyl, while the sextet and quartet must be due to CH_2 groups in the other chain.

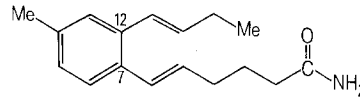
Finally the aromatic substitution pattern was elucidated. Of the 6 possible positional isomers, only 1 was consistent with the observed order of induced shifts of the aromatic protons in (I), (III) and (V). In compounds (I) and (III), $Eu(DPM)_3$ associates with both functional groups but the effect of the amide is greater than either of the oxygen functions at C-13. This results in a higher induced shift for the proton at C-8 compared to the other *ortho* proton at C-11 (see Table). In compound (V), only the amide associates with the complex. The induced shift of the proton at C-8 is still the largest but the shifts of the protons at C-9 and C-11 are now identical which is consistent with their equidistance from the amide. Moreover, the low induced shifts of these two protons confirm their *meta* rather than *ortho* substitution relative to the amide chain.

Protons at C No.		δ (I)	$\Delta\delta^a$ (I)	δ (III)	$\Delta\delta^a$ (III)	δ (V)	$\Delta\delta^a$ (V)
CH_2	2	2.21	2.35	2.21	2.35	2.25	2.35
CH_2	3	1.77	1.94	1.84	1.91	1.79	1.93
CH_2	4	2.24	1.10	2.27	1.08	2.25	1.10
CH=	5	5.94	0.63	5.89	0.63	5.96	0.58
CH=	6	6.68	0.63	6.65	0.62	6.63	0.46
CH=	8	7.27	0.28	7.37	0.25	7.25	0.22
CH=	9	7.01	0.10	7.20	0.09	6.96	0.08
$C-CH_3$	10	2.31	0.07	2.35	0.06	2.29	0.04
CH=	11	7.23	0.23	7.34	0.14	7.25	0.08
	13	4.95	0.51			6.60	0.21
	14	1.68	0.33	2.83	0.13	6.06	0.15
CH_2	15	1.38	0.21	1.68	0.07	2.02	0.06
CH_3	16	0.92	0.11	0.96	0.02	1.08	0.04

[a] $\Delta\delta$ $Eu(DPM)_3$ induced paramagnetic shifts.



(I) R_1=H, R_2=OH, R_3=NH_2
(II) R_1=H, R_2=OAc, R_3=NH_2
(III) R_1+R_2=O, R_3=NH_2
(IV) R_1=H, R_2=OH, R_3=OH



(V)

Compounds (I) and (V) probably arise biosynthetically from a suitably unsaturated 10-methylpalmitic acid precursor by a similar pathway (in this case a 7,12 cyclization would be involved) to that discussed recently[3] for the antibiotic brefeldin A and the biosynthetically related prostaglandins. We have established[4] that antibiotic X-537A is assembled from acetate, propionate and butyrate units, suggesting that in the X-537 co-metabolites (I) and (V), the aromatic methyl arises from a propionate unit rather than a C_1 donor system.

Zusammenfassung. Die Struktur des *trans*-6-[2-(1-hydroxybutyl)-4-tolyl]-hex-5-enamids (I), eines neuen mikrobiellen Metaboliten, wurde durch Protonenresonanz-Spektroskopie unter Verwendung des Verschiebungsreagenz Eu $(DPM)_3$ bestimmt.

T. WILLIAMS, A. STEMPEL, R. H. EVANS JUN., A. JACOBY and J. W. WESTLEY

Chemical Research Department, Hoffmann-La Roche Inc., Nutley (New Jersey 07110 USA), 19 December 1972.

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The Sterols of the Echinoderm, *Ctenodiscus crispatus* Retzius

Echinoderms have been known for some time to contain mixtures of sterols[1], but recent work[2,3] has shown these to be more complex than previously envisaged. We report here the composition of the free sterol fraction obtained from the mudstar *Ctenodiscus crispatus* Retzius, Order Phanerozonia, Family Porcellanasteridae. Unlike other sea stars that have been examined, this species is not carnivorous, but feeds by ingesting from muddy ocean bottoms, on which it lives[4].

Methods. The live animals were blended in chloroform, which was then washed, dried, and chromatographed on a silica-gel column. Visual fractions turned out to contain primarily different classes of metabolites, major ones being neutral glycerides and free sterols. Typically, 87 animals (1425 g net weight) gave 12.1 g of glycerides and 2.72 g of sterols. A clean, crystalline sterol fraction was obtained by chromatography on Florisil (using isooctane/ether, 3:1) and had m.p. 135–138°, $\alpha]_D^{22}$ 0.4 (*c* 1.49 g/100 ml, chloroform). Preparative TLC of the sterol fraction on silica gel ($HF_{254+366}$) containing 20% silver nitrate (4 elutions with chloroform) gave 3 bands, *viz.* Band I (least polar, 85%), Band II (15%) and Band III (most polar, trace quantity), the first 2 of which corresponded primarily to monoenic and dienic sterols. Band III was not examined further.

Results and discussion. Examination of the GLC and the IR-, UV- and NMR-spectra of the sterol fraction, as well as of its monoacetate and monomethyl ether, suggested the presence of a mixture of cholestenols. The mass spectra (*MS*) of the sterols and of their methyl ethers showed quite clearly that the mixture was primarily a series of homologs of 5α-cholest-7-en-3-ols[5,6]. Unusually intense M-2 peaks suggested the presence of dehydrocho-

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