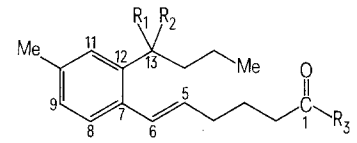
(quintet), respectively, and the relative magnitudes of these shifts indicated that the quintet was due to a CH_2 group β to the amide carbonyl, while the sextet and quartet must be due to CH_2 groups in the other chain.

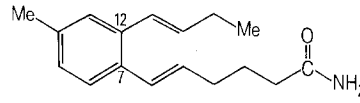
Finally the aromatic substitution pattern was elucidated. Of the 6 possible positional isomers, only 1 was consistent with the observed order of induced shifts of the aromatic protons in (I), (III) and (V). In compounds (I) and (III), $Eu(DPM)_3$ associates with both functional groups but the effect 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.

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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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GLC-MS data on the sterol mixture[a]

Peak Number	Retention time relative to peak a	Abundance, (% of total sterol)	M^+ (amu)	Assignment
			388	5α-cholestan-3β-ol
a	1.00	2.5	386	uncertain, not cholesterol
			384	uncertain
b	1.12	42.0	386	5α-cholest-7-en-3β-ol
c	1.36	8.5	400	24ξ-methyl-5α-cholest-7-en-3β-ol
d	1.43	1.7	—	Uncertain
e	1.67	30.0	414	24ξ-ethyl-5α-cholest-7-en-3β-ol
f	1.95	0.3	428	24ξ-propyl-5α-cholest-7-en-3β-ol
g	1.00	0.3	386	Cholest-5-en-3β-ol
			384	5α-cholesta-7,22-dien-3β-ol
h	1.08	trace	—	Not measured
i	1.20	0.1	398,396	Uncertain, but possibly contains 24ξ-methyl-5α-cholesta-7,22-dien-3β-ol
j	1.32	2.0	398	24-methylene-5α-cholest-7-en-3β-ol
k	1.61	0.6	412	Uncertain, but possibly contains 24ξ-ethyl-5α-cholesta-7,22-dien-3β-ol
l	1.73	6.0	412	24-ethylidene-5α-cholest-7-en-3β-ol
m	1.87	0.5	426,424	Uncertain
n	2.01	5.5	426	24-propylidene-5α-cholest-7-en-3β-ol

[a] Glc on 3% OV-101 at 255 °C; peaks a-f and g-n correspond to results from bands I and II, respectively.

lestenols, and since the peaks at *m/e* 388 or 402 were 8% more intense than expected, a cholestanol was also present. Oxidation of the sterol mixture with CrO_3/acetone, followed by treatment with a trace of base, gave a ketonic material with appropriate spectroscopic properties for 5α-cholest-7-en-3-ones[7], although its UV-spectrum indicated the presence of ca. 0.8% cholest-5-en-3β-ol (cholesterol) in the sterol mixture.

Subjection of the material from Bands I and II to combined GLC-MS confirmed that the separation of Δ^7 monoenes from Δ^7 dienes had been successfully accomplished. Notwithstanding, many GLC peaks still were not homogeneous and consequently complete component identification was not possible. The assignments we have made are shown in the Table. They are based on comparison of the experimentally derived spectra with those reported in the literature[8] and with those obtained from standard materials[9]. The major peaks b, c, e, j, l and n were easily identified (see Table). Significantly intense M+2 ions in the spectra of peaks j, l and n could reasonably be assigned to 24-cholesterol homologs since cholesterol itself has an Rf (in the TLC system used) similar to the Rf of the Δ^7 diene fraction. Peak a contained 5α-cholestan-3β-ol from its molecular ion at *m/e* 388 and its characteristic doublet at *m/e* 234, 233. The *m/e* 386 ion in GLC peak a could not have arisen from cholesterol since the *m/e* 301 ion in the spectrum was not sufficiently intense. The major components in peaks g, i and k possessed parent ions at *m/e* 384, 398, and 412 respectively and all contained a relatively intense *m/e* 300 fragment ion. We have tentatively assigned these components respectively to 5α-cholesta-7,22-dien-3β-ol and its 24-methyl and 24-ethyl homologs.

The gross composition of this sterol mixture is essentially similar to those studied by GOAD[2] and SCHEUER[10]. The presence of small amounts of 5α-cholestan-3β-ol and of Δ^5-sterols confirms GOAD's findings and current suggestions[2] for the biosynthesis of sterols in asteroids. Of interest is the presence of several apparently different, previously unreported, C-30 sterols. Only three C-30 cholestenol homologs have been reported to date[1,11], two being Δ^5 and Δ^7 isomers of a sterol with a cyclopropane-containing side chain and the other being (Z)-24-propylidenecholest-5-en-3β-ol which was found in a local mollusc[11]. Considering the probable biosynthesis of sterols in sea stars, it is perhaps not unexpected that *C. crispatus* should yield the Δ^7 isomer of this mollusc sterol, together with its 24–28 dihydro derivative. An interesting question raised by these results[12] is whether a comparable mixture of Δ^5 sterols can be isolated from the mud on which these sea stars feed.

Zusammenfassung. Aus dem Schlammstern *Ctenodiscus crispatus* wurde ein kompliziertes Gemisch von Sterolen isoliert, die als Homologe des 5α-Cholest-7-en-3β-ols und deren 24-Methylderivate erkannt wurden.

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[7] The mass spectrum of the ketone mixture differed from that of the parent alcohol by 2 units in all the appropriate peaks.

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[9] 5α-cholest-7-en-3β-ol was obtained from Ikapharm Ltd., Ramat-Gan, Israel, and we thank Professor P. J. SCHEUER for a sample of its 24ξ-methyl homolog.

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