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ACANTHELLIN-1, AN UNIQUE ISONITRILE SESQUITERPENE FROM THE SPONGE *ACANTHELLA ACUTA*

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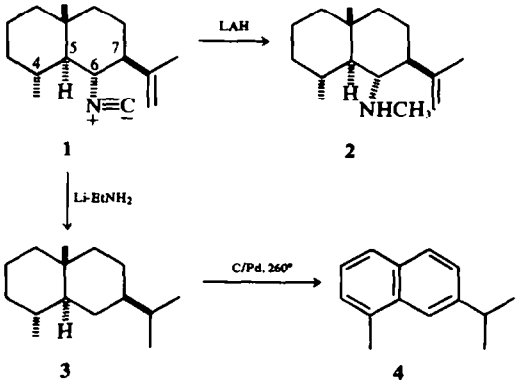
Abstract—A number of unique isonitrile sesquiterpenes have been isolated from the sponge *Acanthella acuta*. One of them acanthellin-1, with antimicrobial activity, is shown to be **1** with a 4-*epi*-eudesmane skeleton.

The isonitrile function is a very rare feature in nature. Until now, only the fascinating metabolite xanthocillin, isolated from *Penicillium notatum*[1] has been described as a natural isonitrile. Very recently a sesquiterpene bearing an isonitrile function, has been isolated from the sponge *Axinella cannabini*.[2] More isonitrile sesquiterpenes have now been obtained in our laboratory from the sponge *Acanthella acuta*, species belonging to the same order *Axinellida*.

Chromatography of the ether-soluble portion from the acetone extracts of the fresh tissues gave three principal fractions, acanthellin-1 (**1**), showing antibacterial activity, acanthellin-2 $C_{16}H_{25}N$, $[\alpha]_D = -24{\cdot}1°$, ν_{max} 2140 cm^{-1} ($-\overset{+}{N}\equiv\overset{-}{C}$) and a fraction consisting of at least three substances which showed IR absorption at 2140 cm^{-1} ($-\overset{+}{N}\equiv\overset{-}{C}$) and a MS ($M^+$/e 231) very similar to those of acanthellins.

The minor components are being investigated and in this paper we report data which establish structure **1** for the major component, acanthellin-1, $C_{16}H_{25}N$, optically-active oil, $[\alpha]_D = -41{\cdot}2°$, showing ν_{max} 2140 (isonitrile) and 895 (exomethylene) cm^{-1}. The presence of an isonitrile function was readily confirmed by conversion of acanthellin-1 into the N-methyl amine (**2**), M^+/e 235; δ N-Me 2·38, while the presence of an exomethylene group is supported by the presence of a typical $=CH_2$ broad singlet at δ 4·85 in the NMR spectrum of **1**. This spectrum also includes signals for a t-Me group (δ 0·85), a sec-Me resonating at relatively low field (δ 1·22, J = 6 Hz), and a vinyl Me (d, J = 1Hz) at δ 1·75. Irradiation at δ 4·85 ($=CH_2$) collapses the doublet at δ 1·75 to a singlet; thus the exomethylene and the vinyl methyl groups comprise an isopropylidene function. This spectral and chemical evidence suggests that acanthellin-1 is a bicyclic sesquiterpene carrying an isonitrile, an isopropylidene and sec and t-Me groups. Lithium-ethylamine reduction of acanthellin-1 (**1**) afforded a saturated hydrocarbon (**3**), $C_{15}H_{28}$, M^+/e 208, $[\alpha]_D = +24{\cdot}1°$, no NMR signals $> \delta$ 1·7, which was converted, in good yield, into eudalene (**4**) by dehydrogenation with palladised charcoal.

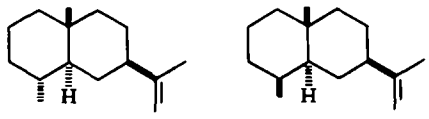


SCHEME 1

Analysis of the 100 MHz NMR spectrum of acanthellin-1 (**1**) and double resonance experiments suggested that the isonitrile group is located at a carbon (C-6) flanked with the isopropylidene-bearing carbon, and with another possessing one H atom, thus ruling out the alternative eremophilane-type structure. In fact, the methine triplet (J = 11 Hz) at δ 3·27 (H-6) is transformed into a doublet with J = 11 Hz by irradiation on the vinylic methine hydrogen at δ 2·15 (H-7). Irradiation at δ 1·20 (H-5) also collapses the triplet at δ 3·27 into a doublet with J = 11 Hz. The values of the coupling constants require a *trans*-diaxial relationship for these three hydrogens and accordingly a *trans*-diequatorial relationship between the isopropylidene and isonitrile groups. Careful measurements of the NMR line width at half-height of the angular Me group ($\Delta_{\frac{1}{2}} = CH_3W_{\frac{1}{2}} - TMSW_{\frac{1}{2}} =$ 0·80 Hz) suggested a *trans*-ring junction.[3] Concerning the stereochemistry of the sec-Me, its relatively low field resonance (δ 1·22) implies that it is *cis* (equatorial) to the isonitrile group (equatorial), thus lying close to this latter and in the plane of the N—C multiple bond (deshielding zone).

The apparent lack of similarity of the NMR properties of the olefin **5**, M^+/e 206, ν_{max} 885 cm^{-1}, δ sec-Me 0·80 (J = 7 Hz), δ t-Me 0·83 in CCl_4, obtained by

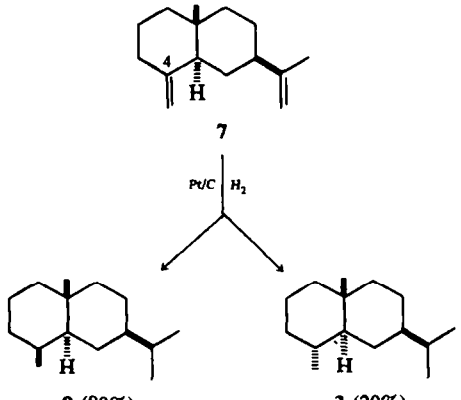
reduction with sodium in liquid ammonia of the natural isonitrile, with those of (∓) 1β, 4aβ-dimethyl-7β-isopropenyl-8aα decahydronaphtalene **6**, δ sec-Me 0·90 (J = 6·6 Hz) and δ t-Me 0·93 in CCl_4, described by Heathcock and Ratcliffe,[4] support the equatorial nature of the C-4 Methyl in acanthellin-1.



5 **6**

The stereochemistry of acanthellin-1 (**1**), suggested by NMR techniques, was confirmed by direct comparison of the hydrocarbon **3** with the epimeric mixture obtained by hydrogenation (Pt/C) of an authentic sample of β-eudesmene (**7**), which resulted in the identification of **3** as 4-*epi*-eudesmane.

Dihydroeudesmol, obtained as the major product in the hydrogenation of β-eudesmol over platinum, was assumed by Barton[5] to have an axial Me group on the grounds that hydrogen should be added to the less hindered face of the molecule. Subsequently Heathcock and Ratcliffe[4] repeated this hydrogenation and found that isomers with axial Me and equatorial Me are produced in the ratio 4:1. We have now hydrogenated β-eudesmene (**7**, Scheme 2) and we also obtained two isomers in a ratio (GLC) of *ca* 4:1. We reasonable assume that the major isomer has an axial Me (**8**); the minor one is consequently the epimer with an equatorial Me at C-4 and this proved to be indistinguishable by GLC in two different systems with the hydrocarbon **3**, obtained by lithium-ethylamine reduction of acanthellin-1. Thus the structure and the



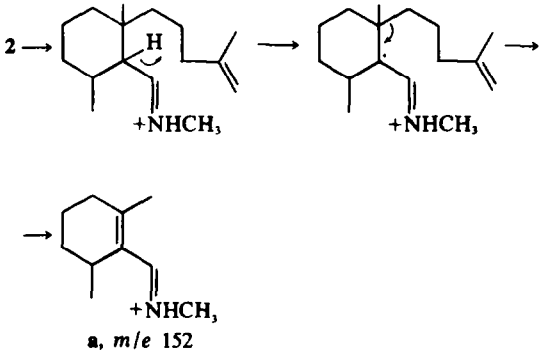
7

8 (80%) **3** (20%)

SCHEME 2

stereochemistry of the natural isonitrile sesquiterpene can be represented as **1**.

The location of the isonitrile group, suggested by the NMR spectrum, is confirmed by the mass spectrum of the N-methylamine derivative **2**, which has the base peak at *m/e* 152 corresponding to fragment **a** (Scheme 3) presumably arising as indicated by Budzikiewicz *et al.*[6] for structurally analogous compounds. The formation of this ion as **a** is supported by deuterium labelling; in the mass spectrum of the N—CD_3 derivative, obtained by LAD reduction of acanthellin-1, the peak at *m/e* 152 is replaced, as expected, by a peak at *m/e* 155.



a, *m/e* 152

SCHEME 3

EXPERIMENTAL

Sponges (*Acanthella acuta*), collected in the Bay of Naples, were obtained from the supply department of the Zoological Station, Naples. IR spectra were recorded on a Perkin-Elmer 257 Infracord spectrophotometer for solns in CCl_4 unless otherwise indicated. NMR spectra were taken at 100 MHz on a Varian HA-100 instrument for solns in CCl_4 unless otherwise indicated (TMS as internal reference). Mass spectra were recorded on an A.E.I. MS-9 instrument with a direct inlet system (70 eV). Rotations were measured in $CHCl_3$ with Perkin-Elmer 141 polarimeter. Elemental analyses were performed by Mr. V. Calandrelli of our laboratory. Silica gel 0·05–0·2 mm (Merck) was used for column chromatography.

Extraction of Acanthella acuta. Fresh sponge (32 g dry after extraction) was extracted with cold acetone (3 × 0·51) for 24 h; after concentration the aqueous residue was extracted with ether (3 × 0·21). The combined ethereal extracts were taken to dryness and the oily residue (4·2 g) was chromatographed on a column of silica (300 g) to give, on elution with light petroleum and increasing proportions of benzene, three fractions. Elution with light petroleum–benzene (8:2) gave acanthellin-2, 162 mg, homogeneous by TLC and GLC (1% OV-1 at 150°) $[\alpha]_D = -24·1°$ (*C*, 5) (Found: C, 83·06; H, 10·90; N, 6·04. $C_{16}H_{25}N$ requires: C, 83·11; H, 10·82; N, 6·06%), *m/e* 231 (M^+), ν_{max} 2970, 2140 (—$\overset{+}{N}\equiv\overset{-}{C}$), 1620 (C=C) cm^{-1}. Elution with light petroleum–benzene (7:3) gave initially a mixture of at least three substances (220 mg) (GLC on 1% OV-1, 150°), ν_{max} 2140 cm^{-1}, *m/e* 231 (M^+) followed by a major fraction consisting of **1** 290 mg homogeneous by TLC and GLC (1% OV-1 at 150°), $[\alpha]_D = -41·2°$ (c, 5) (Found: C, 83·20; H, 10·75; N, 6·05. $C_{16}H_{25}N$ requires C, 83·11; H, 10·82; N, 6·06%), ν_{max} 2970, 2140 (—$\overset{+}{N}\equiv\overset{-}{C}$), 1640

(C=C), and 885 (=CH_2) cm^{-1}, δ 4·85 (2H, bs, =CH_2), 3·27 (1H, t, J = 11 Hz, CH—N), 2·15 [1H, m, —CH—C(CH_3)=CH_2], 1·75 (3H, d, J = 1 Hz, Me—C=C), 1·22 (3H, d, J = 6 Hz, sec-Me) and 0·84 (3H, s, t-Me), *m/e* 231 (M^+, 26%), 216 (M^+—CH_3, 18%), 204 (M^+—HCN, 10%), 189 (M^+—CH_3—HCN, 13%), 188 (13%), 123 (57%), 109 (100%) and 95 (58%).

Lithium aluminium hydride reduction of acanthellin-1 (1). Acanthellin-1 (30 mg) in dry ether (5 ml) was added in portions with stirring to a suspension of LAH (20 mg) in ether (10 ml), and the mixture was stirred for 3 h under reflux. After cooling, water (20 ml) was added, and the mixture was extracted with ether. Evaporation of the ether gave an oily residue, which was applied to a column of silica gel (12 g). Elution with $CHCl_3$—MeOH (9:1) gave 20 mg of 2 (oil). (Found: C, 81·66; H, 12·36, N, 5·98. $C_{16}H_{29}N$ requires: C, 81·70; H, 12·34; N, 5·95%). δ ($CDCl_3$) 4·92 (2H, bs, =CH_2), 3·17 (1H, m, exchangeable with D_2O, NH), 2·75 (1H, t, J = 11 Hz, CH—N), 2·38 (s, N—CH_3), *m/e* 235 (M^+, 13%), 220 (M^+—CH_3, 9%), 152 (a, 100%), 110 (18%) and 84 (45%).

Lithium–ethylamine reduction of acanthellin-1 (1). To a soln of 1 (120 mg) in $EtNH_2$ (10 ml) in a flask equipped with an ice condenser, Li (60 mg, cut in small pieces) was added, and the mixture was stirred at 0° for an additional 1 h after the blue colour developed. A small amount of NH_4Cl was added and the ethylamine was kept *in vacuo*. Finally, water was added and the mixture was extracted with light petroleum. Evaporation of the solvent afforded an oily residue which was chromatographed on silica gel (20 g) to give, by elution with light petroleum, 68 mg of 3, single peak in GLC on 5% SE-30 at 150° and 1% OV-1 at 100°, $[\alpha]_D = +24·1°$ (c, 4), δ methyls at 0·78, 0·84 and 0·90, *m/e* 208 (M^+, 42%), 193 (M^+—CH_3, 48%), 165 [M^+—$CH(CH_3)_2$, 50%], 109 (100%) and 95 (95%). This was indistinguishable in GLC (S% SE-30 at 150° and 1% OV-1 at 100°) from the minor component of the epimeric mixture obtained by hydrogenation (5% PT/C, r.t. and atmospheric pressure for 4 h) of β-eudesmene 7.

Sodium–ammonia reduction of acanthellin-1 (1). To a soln of 1 (80 mg) in liquid NH_3 (15 ml) at −50° under stirring, Na (30 mg) was slowly (2 h) added. A small amount of NH_4Cl was added and the NH_3 was removed *in vacuo*. Finally, water was added and the mixture was extracted with light petroleum. Evaporation of the solvent afforded the oily residue, which was chromatographed on alumina (10 g) impregnated with silver nitrate (3 g) to give, by elution with light in petroleum–benzene (9:1), 35 mg of 5, single peak in GLC on 5% Se-30 at 150° and 1% OV-1 at 100°, M^+/e 206, ν_{max} 885 cm^{-1}, δ 4·62 (2H, bs, =CH_2), 1·70 (3H, d, J = 1 Hz, Me—C=C), 0·83 (3H, s, t-Me), 0·80 (3H, d, J = 7 Hz), sec-Me).

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