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ISOLATION AND STRUCTURE OF AXISONITRILE-1 AND AXISOTHIOCYANATE-1 TWO UNUSUAL SESQUITERPENOIDS FROM THE MARINE SPONGE *AXINELLA CANNABINA*[a]

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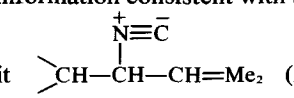
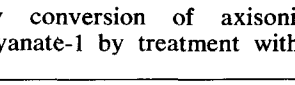
Abstract—Two sesquiterpenoids, axisonitrile-1 and axisothiocyanate-1, have been isolated from the sponge *Axinella cannabina*. On the basis of chemical and physico-chemical evidence structure **1** is suggested for axisonitrile-1 and structure **2** for axisothiocyanate-1.

The only known naturally occurring isonitrile is the antibiotic xanthocillin discovered by Rothe[1] in cultures of *Penicillium notatum* Westling and *Penicillium chrysogenum*. During our studies on the metabolites of Porifera[2] we isolated a sesquiterpenoid isonitrile, axisonitrile-1, from the marine sponge *Axinella cannabina* and describe the assignment of structure **1** to this compound. In addition, we isolated from the same sponge axisothiocyanate-1 (**2**) and the structure determination proved it to be an isothiocyanate strictly related to **1**.

Axisonitrile-1 (**1**). Fresh material was extracted with acetone and the ether soluble fraction, after chromatography on silica gel, afforded **1**, $C_{16}H_{25}N$ (elemental analyses and mass spectrum), m.p. 43–45°, $[\alpha]_D + 22{\cdot}6$.

The presence of the isonitrile group was deduced from the IR (ν_{max} 2130 cm^{-1}) and mass spectra [intense ion at *m/e* 204 ($M^+ - HCN$)]. The NMR spectrum suggests that the isonitrile function is linked to a methine group (δ 3·13, 1H, bm).

Both IR (ν_{max} 3050, 1640 and 895 cm^{-1}) and NMR spectra (δ 4·75, 2H, s) clearly indicate that a $\rangle C{=}CH_2$ group is present in **1**. A further structural feature, revealed by NMR, is the presence of three Me groups, one tertiary (δ 0·99, s) and the other two secondary (δ 0·85 and 1·03, d, J = 6 Hz). The IR data (ν_{max} 1385 and 1375 cm^{-1}) indicates that the two secondary methyls are part of an isopropyl group. These facts strongly suggest that **1** is a bicyclic sesquiterpenoid isonitrile.

Further information consistent with the presence of the unit  (A) was obtained by conversion of axisonitrile-1 into axisothiocyanate-1 by treatment with sulphur at 120°. Since an accurate analysis of NMR spectrum of **2**, as described below, suggests the presence of the unit  it follows that unit A must be present in **1**. This was confirmed by the following experiments: LAH reduction of **1**, afforded the amine **3**, $n_D^{25°}$ 1·4940, $[\alpha]_D + 15{\cdot}5$, M^+ 235 *m/e*; **3** by methylation and subsequent treatment with AgOH gave the corresponding quaternary base which, by thermal decomposition, afforded **4** in high yield, $n_D^{30°}$ 1·5014; $[\alpha]_D - 91{\cdot}7$; M^+ 204 *m/e*.

Compound **4** contains the unit $\rangle CH{-}CH{=}C{=}Me_2$ as shown by its NMR spectrum: δ 4·86 (1H, doublet broadened by long range coupling, J = 9 Hz, H—C_{10}) and 1·64 and 1·52 (6H, singlets broadened by long range coupling, H_3—C_{12} and H_3—C_{13}).

Additional proof for the structure of axisonitrile-1 was provided as follows: Compound **1** afforded **5** [$n_D^{25°}$ 1·4802; $[\alpha]_D + 8{\cdot}5$; M^+ 206 *m/e*; δ 4·65 (2H, m, H_2—C_{15})] by reduction with sodium in liquid ammonia and **6**, axane, ($n_D^{25°}$ 1·4753; $[\alpha]_D - 5{\cdot}5$; M^+ 208 *m/e*) by treatment with lithium in ethylamine.

Ozonization of **5**, followed by decomposition of the ozonide with Na_2SO_3aq, gave ketone **7**, $n_D^{25°}$ 1·4773; $[\alpha]_D + 39{\cdot}1$, M^+ 208 *m/e*. The IR spectrum of **7** (ν_{max} 1707 cm^{-1}) suggests that the keto group is in a 6-membered ring.

Evidence for the position of the keto group in **7** and consequently of the *exo*-methylene in **1** was secured by deuterium exchange of enolisable hydrogens of **7**. The mass spectrum of deuterated compound revealed that three deuterium atoms are incorporated and as a consequence the unit must be present in the 6-membered ring of axisonitrile—1.

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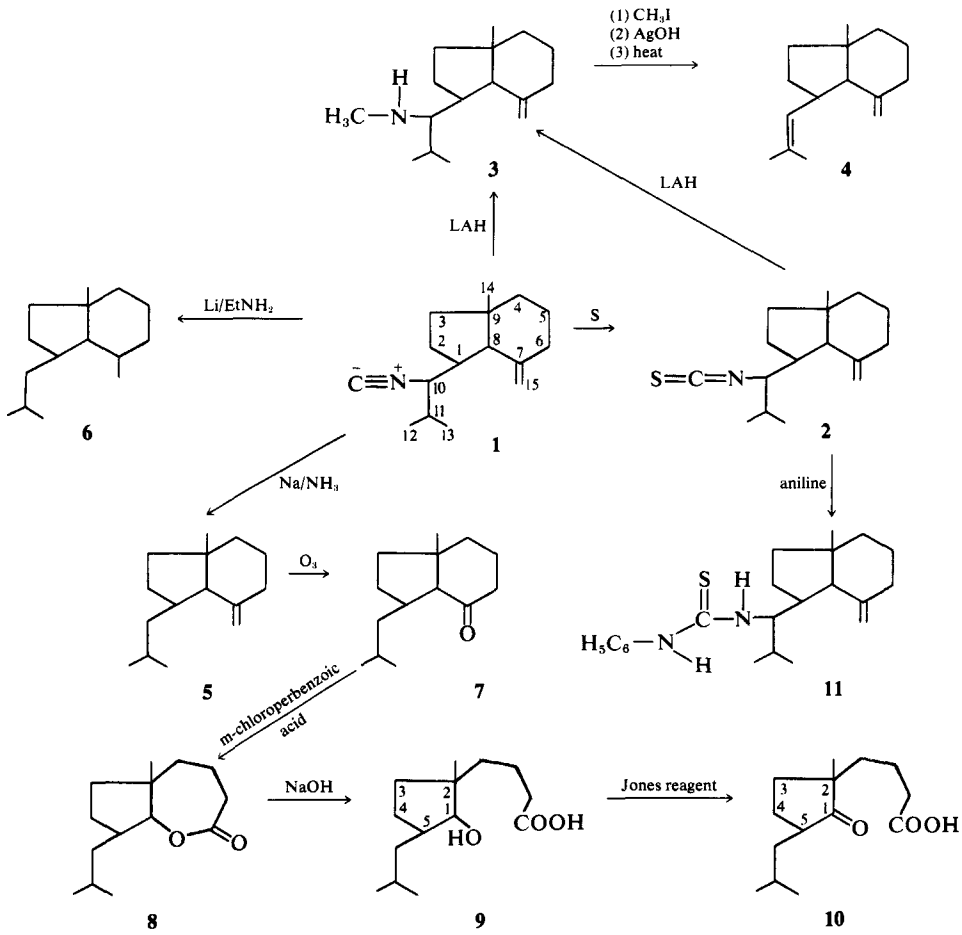

SCHEMA 1

The ketone **7**, by Baeyer-Villiger oxidation, afforded lactone **8**, $n_D^{20°}$ 1·4895; $[\alpha]_D$ −33·6; M^+ 224 *m/e*; ν_{max} 1745 cm^{-1} (CO lactone). In the NMR spectrum a doublet at δ 3·72 (1H, J = 5 Hz, H—C—O—) is present.

Alkaline hydrolysis of **8** gave the hydroxy acid **9**, m.p. 92–94°; $[\alpha]_D$ −36·8; M^+ 242 *m/e*; ν_{max} 1705 cm^{-1}, δ 3·25 (1H, d, J = 6.5 Hz, H—C—OH).

Finally **9**, by treatment with Jones reagent afforded **10**, $n_D^{25°}$ 1·4690; $[\alpha]_D$ −107·0; M^+ 240 *m/e*. The IR spectrum shows a band at 1738 cm^{-1} consistent with the presence of a cyclopentanone system.

Only one hydrogen must be present on the C atoms α to >C=O (C_2 and C_5): in fact, the NMR spectrum of **8** (δ 3·72, 1H, d, H—C—O—) and **9** (δ 3·25, 1H, d, >CH—OH) indicates that in the cyclopentane ring the part structure >CH—C(H)—C(=O)— is present.

In the light of these results the three substituents present on C_2 and C_5 in **8** and **9** and, consequently, in **10** must be a tertiary Me group, an isobutyl group and the acidic residue arising from the oxidative degradation of the 6-membered ring.

Since the isobutyl group, as reported above, is linked to a methyne group, the Me and $-(CH_2)_3COOH$ groups are located on the same C atom.

These considerations, together with all the other results, led us to propose structure **1** for axisonitrile-1.

Axisothiocyanate-1 (**2**). The second compound present in *Axinella cannabina* in smaller amounts was also isolated from the ether soluble fraction of the acetone extract by chromatography on silica

gel. Compound **2** is an oily substance, $C_{16}H_{25}NS$ (elemental analyses and mass spectrum) $n_D^{25°}$ 1·5394; $[\alpha]_D$ +5·9. Spectral data [ν_{max} 3050, 1650 and 895 cm^{-1}; δ 4·78 (2H, bm)] point to the presence of a $\rangle C{=}CH_2$ group. Axisothiocyanate-1 possess three Me groups, one tertiary (δ 0·98, 3H, s) and two secondary (δ 0·89, 3H, d, J = 7 Hz and δ 1·00, 3H, d, J = 7 Hz). The presence of an isothiocyanate function was deduced from the IR (ν_{max} 2120 cm^{-1}), UV (λ_{max} 243 nm, ϵ 2500) and mass spectra [ions at *m/e* 230 (M^+ —HS) and 204 (M^+ —HNCS)]. This was confirmed by treatment of **2** with aniline which gave in high yields thiourea **11** m.p. 63–66°; $[\alpha]_D$ −33·5; M^+ 356 *m/e*. Inspection of the NMR spectrum of **2** also indicated that the isothiocyanate group is linked to a methyne group (δ 3·27, 1*H*, *t*, *J* = 5·5 Hz).

Further analysis of the NMR spectrum of **2** and spin decoupling experiments provided useful information consistent with the presence of the unit $\rangle CH{-}\underset{\displaystyle N{=}C{=}S}{\underset{|}{CH}}{-}CH{=}Me_2$: by irradiation at δ 2·40, the triplet at δ 3·27 (1H, H—C_{10}) collapses into a doublet; by irradiation at δ 2·00 the triplet at δ 3·27 is simplified into a doublet, while the two Me doublets at δ 0.89 and 1·00 (H_3—C_{12} and H_3—C_{13}) collapse into two singlets.

All these facts suggest a close relationship of **2** with axisonitrile-1 (**1**) as proved: Compound **1** by treatment with sulphur at 120° afforded **2**; furthermore **2** by reduction with LAH gave the same amine (**3**) as obtained from **1**. Since the structure of **1** has been established it follows that structure **2** can be assigned to axisothiocyanate-1.

Axisothiocyanate-1, as well as axisonitrile-1, are sesquiterpenes with a skeleton which has not been found before in a naturally occurring compound.

Biogenetically they are interesting molecules; their structures suggest, in fact, new biogenetic pathways for the formation *in vivo* of the carbon skeleton as well as the isonitrile and isothiocyanate functions.

EXPERIMENTAL

The UV and IR (CCl_4 solns) spectra were recorded on a Perkin-Elmer 402 and 157 spectrophotometer. NMR spectra were determined on a Perkin-Elmer R12A and Varian HA-100 spectrometers in CCl_4 solns using TMS as internal reference with δ = 0; s = singlet, d = doublet, t = triplet, m = multiplet, b = broad. Mass spectra were taken on AEI MS 902 instrument. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by Mr. S. De Rosa (Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico del CNR-Arco Felice– Napoli). TLC and PLC separations were effected using glass packed precoated silica gel F_{254} plates (E. Merck). GLC's were run using a Perkin-Elmer 881 instrument with glass columns 2 m × 0·5 cm (flow of nitrogen 30 ml/min).

Sponges (*Axinella cannabina*), collected in the bay of Taranto, were obtained from Stazione di Biologia marina del Salento-Porto Cesareo (dir. Prof. P. Parenzan).

Isolation of axisonitrile-1 (**1**) *and axisothiocyanate*-1 (**2**) *from the sponge* Axinella cannabina. Fresh sponges (500 g, dry after extraction) were extracted 4 times with acetone at room temp for 2 days. The combined extracts (8l) were concentrated under red press and the remaining aqueous residue was extracted with Et_2O (2l in 3 portions). The organic phase was taken to dryness leaving an oily residue (7·6 g), which was chromatographed on a SiO_2 (760 g) column (eluent: 40–70° light petroleum—C_6H_6 8:2). Fractions of 450 ml were collected. Fractions 10–14, on evaporation, afforded mg 370 of **1** gas-chromatographically pure (2·5% SE 30 on chromosorb W at 145°, 162°, 185°); m.p. 43–45°; $[\alpha]_D$ +22·6 (*c* 1, $CHCl_3$); M^+ 231 *m/e*; ν_{max} 3050, 2130, 1640, 1385, 1375, 895 cm^{-1}; δ 4·75 (2H, s, H_2—C_{15}), 3·13 (1H, bm, H—C_{10}), 1·03 (3H, d, J = 6 Hz, H_3—C_{12} or H_3—C_{13}), 0·99 (3H, s, H_3—C_{14}), 0·85 (3H, d, J = 6 Hz, H_3—C_{12} or H_3—C_{13}). (Found C, 83·12; H, 10·75; N, 6·09. Calc. for $C_{16}H_{25}N$ C, 83·05; H, 10·89; N, 6·05%).

Fractions 3–4 were evaporated to dryness and the oily residue (130 mg) was further purified on a SiO_2 (13 g) column using 40°–70° light petroleum as eluent. Fractions of 10 ml were collected. By evaporation of the fractions 10–12, 70 mg of **2** were obtained as an oily product, $n_D^{25°}$ 1·5394; $[\alpha]_D$ +5·9 (*c* 2·5, $CHCl_3$); M^+ 263 *m/e*; ν_{max} 3050, 2120, 1650, 1385, 1375, 895 cm^{-1}; δ 4·78 (2H, bm, H_2—C_{15}), 3·27 (1H, t, J = 5·5 Hz, H—C_{10}, 1·00 (3H, d, J = 7 Hz, H_3—C_{12} or H_3—C_{13}), 0·98 (3H, s, H_3—C_{14}), 0·89 (3H, d, J = 7 Hz, H_3—C_{12} or H_3—C_{13}). (Found C, 72·75; H, 9·83; N, 5·30; S, 12·20. Calc. for $C_{16}H_{25}NS$ C, 72·95; H, 9·57; N, 5·32; S, 12·16%).

LAH reduction of **1** *to* **3**. LAH (600 mg) and **1** (2·4 g) in dry Et_2O (100 ml) were refluxed for 3 h. EtOAc was added to destroy unreacted LAH. After addition of H_2O and extraction with Et_2O, the organic phase was washed, dried and taken to dryness. The residue was purified by column chromatography (SiO_2, 120 g) using Et_2O as eluent. Fractions of 60 ml were collected. From the fractions 7–10, after evaporation of the solvents, 1·1 g of **3** were obtained, M^+ 235 *m/e*; $n_D^{25°}$ 1·4940; $[\alpha]_D$ +15·5 (*c* 2·5, $CHCl_3$); ν_{max} 3380, 3040, 1640, 1380, 1370, 895 cm^{-1}; δ 4·70 (2H, s, H_2—C_{15}), 2·32 (3H, s, N—CH_3), 0·95 (3H, s, H_3—C_{14}), 0·88 (3H, d, J = 6 Hz, H_3—C_{12} or H_3—C_{13}), 0·80 (3H, d, J = 6 Hz, H_3—C_{13}) ($CDCl_3$).

Hofmann exhaustive methylation of **3** *to* **4**. A mixture of **3** (2·2 g), MeI (10 ml) K_2CO_3 (2·5 g) in H_2O (40 ml) was refluxed for 7 h. After cooling, excess MeI was removed *in vacuo* and the soln was extracted repeatedly with $CHCl_3$. The organic phase, after evaporation of the solvent, afforded 3·3 g of the quaternary salt, which, without further purification, was dissolved in MeOH (40 ml) and water (2 ml). After addition of Ag_2O (4 g) the mixture was stirred for 2 h at room temp; the ppt was removed by filtration and washed with $CHCl_3$. The filtrate, taken to dryness, gave 2·97 g of crude quaternary base, which was heated at 160°–180° for 30 min.

The distillate was dissolved in Et_2O (10 ml), dried over $CaSO_4$ and evaporated to drynesss. The residue (mg 900) was purified by PLC (8 plates) using 40–70° light petroleum as eluent. The band R_f 0·7 (UV light), after elution with Et_2O, gave mg 530 of **4**, $[\alpha]_D$ −91·7 (*c* 2·7, $CHCl_3$); $n_D^{30°}$ 1·5014; M^+ 204 *m/e*; ν_{max} 1645 and 890 cm^{-1}; δ 4·86 (1H, bd, J = 9 Hz, H—C_{10}), 4·52 (2H, m, H_2—C_{15}), 0·95 (3H, s, H_3—C_{14}) and 1·64 and 1·52 (each 3H, bs, H_3—C_{12} and H_3—C_{13}).

Reduction of **1** *with* Na/NH_3 *to* **5**. To a soln of **1** (300 mg) in liquid NH_3 (25 ml) and Et_2O (25 ml) at $-45°$ under stirring Na was slowly (2 h) added. Excess Na was destroyed with a little NH_4Cl and the resultant mixture was taken to dryness. The residue, after additon of H_2O, was extracted with 40–70° light petroleum and the organic phase was evaporated to dryness to give 290 mg of an oily product, which was chromatographed on a SiO_2 (10 g) column (eluent 40–70° light petroleum). Fractions 1–3 (75 ml), evaporated to dryness, gave 224 mg of **5** gas-chromatographically pure (2·5% SE 30 on chromosorb W at 118° and 128°); M^+ 206 *m/e*; $n_D^{25°}$ 1·4802; $[\alpha]_D$ + 8·5 (*c* 1, $CHCl_3$); ν_{max} 3050, 1640, 1380, 1375, 890 cm^{-1}; δ 4·65 (2H, m, H_2—C_{15}), 0·95 (3H, s, H_3—C_{14}), 0·85 (3H, d, J = 6 Hz, H_3—C_{12} or H_3—C_{13}), 0·80 (3H, d, J = 6 Hz, H_3—C_{12} or H_3—C_{13}).

Reduction of **1** *with* $Li/EtNH_2$ *to axane* (**6**). To a soln of **1** (145 mg) in anhyd $EtNH_2$ (7 ml), Li (60 mg) was slowly added at 16°. After 90 min a little NH_4Cl was added and the $EtNH_2$ was evaporated. After addition of H_2O (10 ml) the suspension was extracted with 40–70° light petroleum; the organic phase was washed with H_2O, dried over $CaSO_4$ and taken to dryness.

The residue (128 mg) was chromatographed on $SiO_2/AgNO_3$ (7:3; 12 g) column (eluent 40–70° light petroleum-Et_2O 49:1). Fractions of 10 ml were collected. Fractions 11–20, after removal of the solvent, afforded 35 mg of **6**, gas-chromatographically pure (2·5% SE 30 on chromosorb W at 128° and 150°); M^+ 208 *m/e*; $n_D^{25°}$ 1·4753; $[\alpha]_D$ − 5·5 (*c* 2, $CHCl_3$); δ 0·97 (3H, s, H_3—C_{14}), 0·90 (3H, d, J = 7 Hz, H_3—C_{15}; irradiation at δ 1·75 collapses this doublet to a singlet), 0·86 (6H, d, J = 6 Hz, H_3—C_{12} and H_3—C_{13}; irradiation at δ 1·56 collapses this doublet to a singlet).

Ozonolysis of **5** *to* **7**. Ozonized O_2 (2% O_3) was passed through a soln of **5** (2·2 g) in MeOH—EtOAc (1:1, 100 ml) at − 40° for 2 h. The ozonide was decomposed with a saturated aqueous soln of Na_2SO_3 (1 g) at 40° for 30 min. After removal of MeOH and EtOAc under red press, the suspension was extracted with 40–70° light petroleum. The organic phase was dried over $CaSO_4$ and taken to dryness. The residue (1·8 g) was chromatographed on a SiO_2 (50 g) column using the following solvent systems: n-hexane-C_6H_6 7:3 (360 ml), n-hexane-C_6H_6 6:4. Fractions of 40 ml were collected. Fractions 23–46, after removal of the solvents, gave **7** (750 mg), M^+ 208 *m/e*; $[\alpha]_D$ + 39·1 (*c* 3, $CHCl_3$); $n_D^{25°}$ 1·4773; ν_{max} 1707, 1380, 1375 cm^{-1}; δ 1·07 (3H, s, H_3—C_{14}), 0·84 (6H, d, J = 6 Hz, H_3—C_{12} and H_3—C_{13}).

Deuteration of enolisable hydrogens of **7**. Compound **7** (5 mg), Na (15 mg), D_2O (0·5 ml) and MeOD (0·5 ml) were heated at 70° for 48 h in a sealed tube. After removal of MeOD *in vacuo*, the suspension was diluted with D_2O, acidified with N DCl in D_2O and extracted with Et_2O. The organic phase was taken to dryness to give 4 mg of **7**-d_3 of 91% isotopic purity, M^+ 211 *m/e*.

Baeyer-Villiger oxidation of **7** *to* **8**. A soln of **7** (240 mg) and *m*-chloroperbenzoic acid (300 mg) in $CHCl_3$ (8 ml) was refluxed for 5 h. After evaporation of the solvent, the residue was dissolved in Et_2O (30 ml). The soln was washed repeatedly with 2N Na_2CO_3 and then with H_2O. After evaporation of Et_2O the residue was chromatographed on a SiO_2 (15 g) column using the following solvents: C_6H_6 (150 ml), C_6H_6—Et_2O 9:1. Fractions of 50 ml were collected. The fractions 23–24, taken to dryness, gave mg 167 of **8**, $[\alpha]_D$ − 33·6 (*c* 2, $CHCl_3$); $n_D^{20°}$ (1·4895; M^+ 224 *m/e*; ν_{max} 1745 cm^{-1}; δ 3·72 (1H, d, J = 5 Hz, H—C(—)—O—), 0·98 (3H, s, *tert.* CH_3), 0·92 (6H, d, —CH(CH_3)(CH_3)).

Alkaline hydrolysis of **8** *to* **9**. To a soln of **8** (120 mg) in dioxane (2 ml) 10% Na_2CO_3aq (8 ml) was added. After refluxing for 2 h the soln was washed with Et_2O, acidified with 2N HCl to pH 4 and extracted with Et_2O. The ethereal extract was washed with H_2O, dried over $CaSO_4$ and evaporated to dryness to give 94 mg of **9**, which was crystallized from 60–80° light petroleum, m.p. 92–94°; $[\alpha]_D$ − 36·8 (*c* 2·5; $CHCl_3$); M^+ 242 *m/e*; ν_{max} 1705 cm^{-1}; δ 3·25 (1H, d, J = 6·5 Hz, H—C(—)—OH, 0·95 (3H, s, tert Me), 0·86 (6H, d, J = 6 Hz —CH=Me_2) ($CDCl_3$).

Oxidation of **9** *to* **10** The hydroxyacid **9** (60 mg) in acetone (5 ml) was treated with Jones reagent for 30 min at room temp. Following the usual work-up **10** (40 mg) was obtained and was purified by PLC (eluent C_6H_6—Et_2O 1:1, R_f 0·4), $n_D^{25°}$ 1·4690; $[\alpha]_D$ − 107·0 (*c* 0·7, $CHCl_3$); ν_{max} 1738 and 1705 cm^{-1}.

Treatment of **2** *with aniline to obtain* **11**. Compound **2** (50 mg) and excess aniline were kept at room temp for 24 h. After dilution with H_2O, the suspension was extracted with Et_2O (50 ml in 3 portions). The combined ethereal extracts, after washing with H_2O, were dried and taken to dryness. The residue (57 mg) was chromatographed on PLC (eluent C_6H_6—Et_2O 9:1). The band R_f 0·7 (UV light) was eluted with Et_2O to give 45 mg of **11**, m.p. 63–66°; $[\alpha]_D$ − 33·5 (*c* 3, $CHCl_3$); M^+ 356 *m/e*; ν_{max} 3380, 3050, 1635, 895 cm^{-1}; δ 7·22 (5H, m, aromatic protons), 4·57 (2H, m, H_2—C_{15}), 0·95 (3H, d, J = 6 Hz, H_3—C_{12} or H_3—C_{13}), 0·89 (3H, s, H_3—C_{14}), 0·72 (3H, d, J = 6 Hz, H_3—C_{12} or H_3—C_{13}).

Treatment of **1** *with sulphur to obtain* **2**. Compound **1** (200 mg) and S (70 mg) were heated at 120° for 16 h; after addition of 40–70° light petroleum (30 ml) and filtration, the soln was taken to dryness and the residue was purified by PLC (2 plates) (eluent: 80–100° light petroleum). The band R_f 0·5, eluted with C_6H_6, gave 100 mg of **2**.

LAH reduction of **2** *to* **3**. Compound **2** (50 mg) was reduced with the experimental conditions used for **1**. Working up as previously described afforded a compound, which was identified as **3** by $n_D^{25°}$, $[\alpha]_D$, and chromatographic (TLC in C_6H_6—Et_2O 8:2) and spectral (IR, NMR and MS) properties.

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