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CO-OCCURRENCE OF TWO TERPENOID ISOCYANIDE-FORMAMIDE PAIRS IN A MARINE SPONGE (*HALICHONDRIA* SP.)

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Abstract—From a marine sponge (*Halichondria* sp.) we have isolated amorphane sesquiterpenoids that are substituted at C-10 by isocyanide (**1**), formamide (**2**), and isothiocyanate (**3**), and diterpenoids that are 3,7,11,15-tetramethyl-1,6,10,14-hexadecatetraenes (geranyllinaloyl) bearing isocyanide (**13**), formamide (**14**), and isothiocyanate (**16**) functions at C-3.

During a screening program for bioactive constituents of marine sponges that was initiated in our laboratory by T. R. Erdman† we examined the organic extract of *Halichondria* sp.‡ which was active against *Staphylococcus aureus*. Spectral studies of the crude oily residue revealed an intriguing infrared band at 2130 cm^{-1}, suggestive of the rare isocyanide function. Up to that time only one naturally occurring isocyanide, the mold metabolite xanthocillin, had been reported.[1] Since then, Italian workers have isolated three new sesquiterpenoid isocyanides, all from marine sponges.[2-4] Work-up of our extract confirmed the suspected isocyanide function in a sesqui- and in a diterpenoid constituent. Each substance was accompanied by a corresponding formamide, an isothiocyanate, and by nonfunctionalized hydrocarbons. This paper describes the details of this work, which was the subject of two preliminary communications.[5,6]

Preparative TLC of the organic sponge extract initially yielded five fractions, in order of increasing polarity: two hydrocarbon fractions, an isothiocyanate, an isocyanide, which possessed the antimicrobial activity, and at the origin a formamide fraction. The less polar hydrocarbon fraction was sesquiterpenoid and could be further separated into two equal components by GLC. The second fraction was the diterpene. The third TLC band, the isothiocyanate, could be further fractionated only after reaction with aniline, which left the severely hindered sesquiterpenoid constituent unreacted. The fourth (isocyanide) TLC band was cleanly separable into sesqui- and diterpenoid constituents by further TLC. The last (formamide) band was invariably contaminated with equal quantities of long chain aliphatic material, which could be removed only after LAH reduction to the two corresponding N-methylamines.

Spectral (NMR, mass) examination of the pure constituents thus obtained made it apparent that each of the two terpenoid series was based on a single carbon skeleton and that the members in each series differed only in the nature of the functional groups. We proved this by interconversion of the isocyanides with the formamides (aq HOAC) and vice versa (benzenesulfonyl chloride, pyridine), and by transforming the isocyanides into the isothiocyanates by heating with sulfur.

The sesquiterpenoids

We established the carbon framework, the site of functionalization and stereochemistry of the sesquiterpenoids (**1-3**) by several degradations which are outlined in Chart 1. The isocyanide function of **1**, $C_{16}H_{25}N$, m.p. 40–42°, ν_{max} 2130 cm^{-1}, was clearly tertiary because of

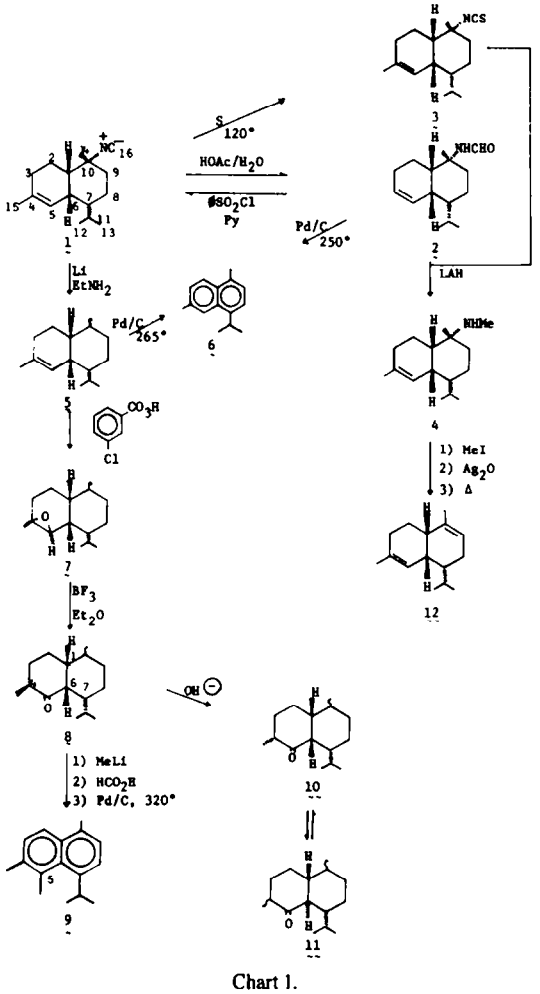

Chart 1.

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absence of PMR signals between δ 2·5 and 5·0, which would have been expected if the isocyanide were secondary or primary. The CMR signal of the functionalized carbon (C-10) is a triplet at δ 57·6 coupled to ^{14}N (J = 4 ± 1 Hz)[7] and becomes a broad singlet in the off-resonance spectrum. C-10 also bears a Me group (δ 1·42) which is weakly coupled (t, J = 1·5 Hz)[8] to ^{14}N of the isocyanide. Other diagnostic spectral features included Me doublets at δ 0·91 and 0·97 (J = 6·5 Hz) assigned to an isopropyl group, a broad signal at δ 1·59 for Me attached to an olefin, and a broad one proton singlet in the olefinic region (δ 5·3). This resonance exhibits W_h coupling of 6 Hz that sharpens to 3·5 Hz on irradiation of the δ 1·59 Me signal.

We showed that our *Halichondria* sesquiterpenoids had a cadalene (**6**) skeleton by Pd/C treatment of formamide **2** or, in better yield, by defunctionalization (Li, $EtNH_2$)[9,10] of **1** to **5**, followed by Pd/C treatment of **5**.

This degradation of **1** to cadalene (**6**) can accommodate an alternate structure, a 9,10-olefin functionalized at C-4. However, lack of multiplicity of the PMR signal at δ 5·3 favored the functional group arrangement as in **1**. We proved this by transforming **5** to epoxide **7**, which has PMR singlets at δ 1·25 (C-4 Me) and at δ 2·52 (proton at C-5); this lack of multiplicity points to a dihedral angle of about 90° between C-5 and C-6 protons. Epoxide **7**, on treatment with boron trifluoride etherate led to ketone **8**, m.p. 50–52°, ν_{max} 1710 cm^{-1}, PMR doublet (J = 7 Hz) at δ 1·18 (C-4 Me) and triplet (J = 4 Hz) at δ 2·95 (C-6 proton). Alkylation of **8** with methyllithium, followed by dehydration and dehydrogenation furnished 5-methylcadalene (**9**) of known structure.

Ketone **8** not only served to place the functionality unambiguously at C-10, but also provided key evidence for the stereochemistry assigned to C-1, C-6 and C-7. When the signal at δ 1·55 (C-1 proton) in the PMR spectrum of **8** is irradiated, the triplet at δ 2·95 (C-6 proton) collapses to a doublet (J = 4 Hz). This small equal coupling of H-6 with H-1 and H-7 is consistent only with the coupling of axial-equatorial protons in a *cis*-decalone system,[11] as *trans* diaxial coupling should be about 10 Hz. On the reasonable assumption that the bulky C-7 isopropyl has equatorial conformation, the *Halichondria* sesquiterpenes possess amorphane stereochemistry at C-1, 6 and 7. The very small coupling between H-5 and H-6 that is observed in compounds **1–5** is in agreement with the sesquiterpenes of the amorphane configuration since models of these compounds show that the dihedral angle between these protons is close to 90°. Compounds having the alternate *cis*-decalone configuration and possessing a cadalene skeleton, the muurolanes, which are epimeric with the amorphanes at C-7, have a small dihedral angle between H-5 and H-6 and a 4–5 Hz coupling is observed.[12] Both amorphane enantiomers are known natural products,[13,14] which may be distinguished by CD measurements, as the octant rule predicts a positive Cotton effect for **8**.[14] We confirmed the stereochemical assignment by observing a positive Cotton effect for **8** (Φ_{302} + 4173, θ_{302} + 9264), and by partial epimerization of **8** at C-6. Treatment of **8** with base gave a mixture of the *cis* and *trans* fused decalones **10** and **11**,[15] exhibiting a weak positive (Φ + 191) and a somewhat stronger negative (Φ − 407) Cotton effect for **10** and **11** respectively.[14] The PMR spectrum of the mixed decalones lacks the signals at δ 2·95, 1·18, 0·83 and 0·73 present in **8**, but instead has a triplet at δ 2·75 (J = 4 Hz), which integrates for one third of a proton and a small pair of isopropyl doublets at δ 0·74 and 0·64 (J = 6·5 Hz). These signals are generated by *cis*-ketone **10** (about 30% of the mixture) and result from epimerization in **8** of the axial Me at C-4, which is a consequence of stereospecific epoxidation and rearrangement reactions in the synthetic sequence **5–8**. All four Me groups in **11** give rise to a series of doublets between δ 0·85 and 0·95.

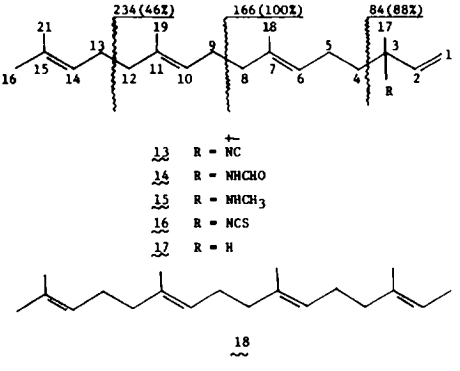
The remaining stereochemical assignment, the configuration at C-10, was deduced from the results of a Hofmann degradation on N-methylamine **4**, which we prepared from formamide **2**. Methylation and treatment with silver oxide furnished a quaternary base, which when heated at 165° yielded a mixture of hydrocarbons. This mixture has only weak IR absorption and no PMR signals appropriate for an exocyclic double bond. It must therefore be a mixture of Δ^1 and Δ^9 olefins. These olefins can only have arisen from an axial amine by diaxial elimination. By analogy, the quaternary ammonium base of 1-methylcyclohexylamine, which doubtless has an equatorial amine group, yields 97% methylenecyclohexane under similar conditions.[16] This reaction sequence proves the stereochemical assignments as shown in **1**. Finally, when an authentic sample of (+)-α-amorphene (zizanene, **12**) was coinjected with the Hofmann mixture, its GLC peak coincided with the major component.

The isothiocyanate fraction on treatment with aniline at room temperature and preparative TLC furnished pure **3**, $C_{16}H_{25}NS$, ν_{max} 2250, 2100 cm^{-1}.[17] The PMR spectrum of **3** is very similar to that of **1**. The methyl group at C-10 appears as a singlet at δ 1·40, while in **1** it is a weakly coupled triplet at δ 1·42. Compound **3** on LAH reduction yielded the 10-methylamino derivative (**4**), identical with **4** derived from natural **2** or from **2** that had been prepared from **1**.

The badly contaminated formamide fraction could be rigorously purified only by LAH reduction of **2** to **4**. Pure formamide (**2**), however, was prepared from **1**.

The diterpenoids

We separated the diterpene isonitrile (**13**) from **1** by TLC as an oil, *m/e* 299 (M^+ 23%) for a composition of $C_{21}H_{33}N$. The isonitrile band is observed in the IR spectrum at 2140 cm^{-1} with vinyl absorptions at 990 and 930 cm^{-1}. The CMR spectrum displayed signals at 155·6 ppm for the isonitrile carbon and an off-resonance singlet at 62·5 ppm for the function C-3, in addition to **8** olefinic resonances, ranging from 114·0 to 138·1 ppm, consistent with an acyclic tetraene. The PMR spectrum of **13** showed singlets (12 protons) at δ 1·59–1·67 assigned to 4 allylic Me groups and a 3 proton triplet (J = 1·5 Hz) at δ 1·47 which we assigned to the Me at C-3 in full analogy with parallel observations in the PMR spectrum of **1**. A



13 R = NC
14 R = NHCHO
15 R = $NHCH_3$
16 R = NCS
17 R = H

18

broad singlet at δ 1·99 (5 allylic methylenes), a series of vinyl multiplets (3 protons) between δ 5·1–5·5, 3 other olefinic protons as a broad peak at δ 5·0, and an unresolved 2 proton resonance for the C-4 methylene near δ 1·2 completed the NMR spectrum. These data coupled with the lack of conjugation (UV end absorption only) suggested a structure for this sponge metabolite of 3-isocyano - 3,7,11,15 - tetramethyl - 1,6,10,14 - hexadecatetraene (**13**), which is the isocyanide analogue of the known[18] jasmine constituent geranyllinalool. Further confirmation of the postulated structure was gained by ozonolysis and reductive work-up of **13** leading to the 2,4-DNPH derivatives of formaldehyde, acetone, and 4-ketopentanal.

Hydrolysis of **13** in acetic acid gave the formamide **14** and LAH reduction of **14** yielded the N-methylamine **15**. The mass spectrum of **15** showed, in addition to the molecular ion at *m/e* 303 (11%), major fragmentation resulting from allylic cleavage as shown.

Reduction of **13** with lithium in ethylamine resulted not in **17** but in an unexpected isomeric hydrocarbon **18** (M^+ *m/e* 274). Its PMR spectrum exhibits only signals for olefinic protons, allylic methylenes and allylic methyls. Formation of **18** may be rationalized by rearrangement of the initially formed allylic radical in the metal-amine reduction of **13**.

Formamide **14** is also a sponge constituent, but is cleanly separable from **2** only after treatment of the mixture with benzenesulfonyl chloride, leading to **13** and **1**. Similarly, isothiocyanate **16** (M^- observed at *m/e* 331) can be removed from its sesquiterpenoid counterpart **3** only after treating the mixture with aniline which left the more hindered **3** unreacted.

Our isolation from *Halichondria* sp. of two terpenoid isocyanide-formamide pairs carries the implication that in this sponge the formamides are the biogenetic precursors of the isocyanides. In the earlier xanthocillin case an attempt to demonstrate by radiofeeding experiments the biosynthetic origin of the mold metabolite had failed.[19]

EXPERIMENTAL

IR spectra were recorded on a Beckman IR-10 spectrophotometer in CH_2Cl_2. PMR and CMR spectra were taken at 100 MHz on Varian HA-100 and XL-100 spectrometers in CCl_4 and $DCCl_3$ respectively, with TMS ($\delta = 0$) as an internal standard s, singlet; d, doublet; t, triplet; m, multiplet; b, broad. The ^{13}C off-resonance multiplicities are shown in parentheses. UV and CD spectra were taken on Cary 14 and Cary 61 spectrophotometers in 95% EtOH and anhyd MeOH respectively. Mass spectra were recorded on a Hitachi RMU-6D instrument with a direct inlet system at 20 eV. Rotations were measured with a Bendix Ericsson ETL-NPL automatic polarimeter type 143A in CCl_4. Gas chromatography was performed with 6′ × 1/8″ 3% OV-17 on Gas Chrom Q and 6′ × 1/8″ 3% SE-30 on Gas Chrom Q stainless steel columns. Elemental analyses were performed by the University of California Chemical Analytical Services, Berkeley, California. Silica gel HF 254 + 366 (EM Laboratories) was used for thin layer chromatography. M.ps were taken on a Fisher–Johns apparatus and are uncorrected. Spectral (NMR, IR, MS) comparison established identity of all interconversion products.

Sponge extraction. Halichondria sp. was collected by trawling at a depth of 200 m off the north shore of Oahu and frozen prior to extraction. The sponge (200 g dry after extraction) was homogenized with MeOH in a blender and the solvent replaced twice at 24 hr intervals. The aqueous concentrate was extracted with ether which was dried ($MgSO_4$) and taken to dryness to yield 4·6 g of oil.

Separation and purification. TLC (silica gel, hexane, visualization with long wavelength UV light) gave 5 major fractions. A sesquiterpene hydrocarbon fraction (42 mg), was isolated at R_f 0·7; it gave two equal peaks on GLC (3% Se-30, 95°), and at R_f 0·6 a diterpene hydrocarbon (42 mg) *m/e* 272. An isothiocyanate fraction (200 mg) was isolated at R_f 0·5, an isocyanide fraction, 850 mg, at R_f 0·3, and an impure formamide fraction 1·4 g obtained from the origin. The isocyanide fraction was purified by TLC (silica gel, CH_2Cl_2–hexane, 1:1) to give **1** at R_f 0·5 (480 mg) and **13** at R_f 0·6 (120 mg).

Isocyanide **1** is a solid m.p. 40–42°, $[\alpha]_D$ − 75° (*c* 5·0, CCl_4); ν_{max} 2130, 1385, 1375 cm^{-1}; PMR δ 5·3 bs, 1·63 s, 1·42 t (J = 1·5 Hz), 0·97 d (J = 6·5 Hz), 0·91 d (J = 6·5 Hz); CMR, ppm 155·4 t (J = 4 ± 1 Hz) (s) [C-16], 136·8 (s) [C-3], 118·8 (d) [C-4], 57·8 t (J = 4 ± 1 Hz) (s) [C-10], 48·4 (d), 42·4 [2 carbons] (d, t), 36·0 (d), 29·8 (q), 28·5 (q) 27·2 (d), 24·0 (t), 23·3 (t), 22·5 (t), 21·6 (q), 20·5 (q); *m/e* 231 (M^+ 45%), 204 (–HCN 100%), 188 (-isopropyl 52%), 161 (–HCN -isopropyl 74%). (Found: C, 82·86; H, 10·75; N, 5·96. Calc. for $C_{16}H_{25}N$: C, 83·05; H, 10·89; N, 6·05%).

Isocyanide **13** is an oil, $[\alpha]_D$ + 15° (*c* 2·8, CCl_4; ν_{max} 2140, 990, 930 cm^{-1}; δ 5·1–5·5 several m, 5·0 b, 1·99 bs, 1·67 s, 1·62 s, 1·59 s, 1·47 t (J = 1·5 Hz), 1·27 m; ppm 155·6 t (J = 4·5 ± 1 Hz) (s) [isocyano carbon], 138·1 (d) [C-2], 136·2 (s), 134·8 (s), 131·0 (s), 123·9 [2 carbons] (d), 122·3 (d), 114·0 (t) [C-1], 62·5 t (J = 4·5 ± 1 Hz) (s) [C-3], 41·5 (t), 39·7 (t), 29·7 (t), 28·4 (q), 26·8 [2 carbons] (t), 25·6 (q), 22·8 (t), 17·7 (q), 16·0 [2 carbons] (q); *m/e* 299 (M^+ 23%), 284 (–CH_3 100%), 272 (–HCN 23%), 230 (23%), 163 (49%), 121 (51%), 81 (46%), 69 (49%).

The isothiocyanates. This fraction (200 mg) was refluxed with freshly distilled aniline (200 mg) in benzene for 5 hr. TLC (silica gel, hexane) gave pure **3** as an oil, 120 mg, $[\alpha]_D$ − 63° (*c* 7·4, CCl_4); λ_{max} 214 (1800), 243 (940) nm; ν_{max} 2250, 2100 cm^{-1}; δ 5·25 bs, 1·70 bs, 1·40 s, 0·94 d (J = 6 Hz) and 0·88 d (J = 6 Hz); *m/e* 263 (M^+ 100%), 230 (–SH 22%), 204 (–HSCN 51%), 161 (–HSCN -isopropyl 43%). (Found: C, 73·12; H, 9·72; N, 5·15; S, 12·04. Calcd. for $C_{16}H_{25}NS$: C, 72·95; H, 9·53; N, 5·32; S, 12·16%).

Hydrolysis of **1** *to* **2.** To **1** (112 mg), 99·7% glacial AcOH (10 ml) was added and the soln allowed to stand 18 hr. The AcOH was evaporated under reduced pressure to give a 2:1 mixture of *trans* and *cis* formamide **3**, oil, $[\alpha]_D$ − 50° (*c* 5·1, CCl_4); ν_{max} 3380, 1680, 1510, 1450, 1390, 1385 cm^{-1}; δ 8·00 d (J = 12 Hz) [ –H *trans*], 7·91 d (J = 2 Hz) [–H *cis*], 5·46 bs [H-5], 1·60 bs [C-15], 1·52 s [C-14 *cis*], 1·42 s [C-14 *trans*], 0·98 d (J = 6 Hz) and 0·90 d (J = 6 Hz) [isopropyl]; *m/e* 249 (M^+ 65%), 234 (–CH_3 58%), 204 (–NH_2CHO 100%), 161 (–NH_2CHO-isopropyl 49%).

Conversion of **1** *to* **3.** A mixture of **1** (105 mg) and S (204 mg) was heated at 120° for 16 hr. Extraction with CH_2Cl_2 followed by TLC gave 37 mg **3**.

Dehydration of **2** *to* **1** *and* **14** *to* **13.** To the crude formamide fraction (200 mg) (*ca.* 50% formamide) in pyridine (10 ml), benzenesulfonyl chloride (1 g) was added and the soln allowed to stand at room temp. for 3 hr. After dilution with water, the suspension was extracted with ether and the ether layer washed with water, then dried. TLC gave 28 mg of **1** and 8 mg of **13**.

Lithium ethylamine reduction of **1** *to* **5.** To **1** (175 mg) in freshly distilled anhyd ethylamine (50 ml), Li (*ca.* 50 mg) was added in small pieces until the soln developed a blue color. After stirring for 1 more hr, a small amount of NH_4Cl was added and the amine was allowed to evaporate. Water was added and the suspension extracted with CH_2Cl_2 which was dried and then evaporated. TLC (silica gel, hexane, UV) gave 94 mg of **5**, R_f 0·7, oil, $[\alpha]_D$ + 11 (*c.* 9·4, CCl_4); δ 5·15 bs, 1·61 bs, 0·93 d (J = 6 Hz), 0·90 d (J = 6 Hz), 0·87 d (J = 6 Hz). GLC (3% OV-17, 95°) showed > 99% purity.

Dehydrogenation of **2** *and* **5** *to cadalene,* **6.** A mixture of **5** (37 mg) and 10% Pd/C 50 mg was heated at 265° under N_2 for 1 Hr. Extraction of the mixture with CH_2Cl_2 and TLC (silica gel, hexane, UV) gave 11 mg cadalene, **6**, R_f 0·6; δ 1·35 d (J = 7 Hz), 2·48 s, 2·54 s, 3·65 septet (J = 7 Hz), 7·08 m, 7·75 m; picrate m.p. 113–115° (lit.[20] 115°).

A mixture of **2** (100 mg) and 10% Pd/C (100 mg) was heated at 250° for 6 hr under N_2. Extraction and TLC gave 10 mg cadalene.

Epoxidation of **5** *to* **7.** To a soln of **5** (61 mg) in CH_2Cl_2, *m*-chloroperbenzoic acid (120 mg) was added and the soln allowed

to stand at room temp. 18 hr. The solvent was removed and the residue shaken with satd $NaHCO_3$ aq. The suspension was extracted with CH_2Cl_2, which in turn, was extracted with dil ferrous sulfate soln, then water, and dried. The solvent was removed and the residue purified by TLC (silica gel, hexane-benzene 9:1, UV) to give 35 mg **7**, R_f 0·2; δ 2·52 s, 1·25 s, 1·05 d (J = 6 Hz), 0·98 (J = 6 Hz), 0·87 d (J = 6 Hz).

Rearrangement of **7** *to* **8**. To a soln of **7** (35 mg) in ether (2 ml), freshly distilled BF_3 etherate (60 mg) was added and the soln allowed to stand 6 hr. The solvent was removed and the residue purified by TLC (silica gel, hexane-benzene 9:1, UV) to give 23 mg **8**, R_f 0·3; m.p. 50–52°; $[\alpha]_D$ + 85 (*c* 2, CCl_4); ν_{max} 1710 cm^{-1}; δ 2·95 t (J = 4 Hz), 1·18 d (J = 7 Hz), 0·87 d (J = 6 Hz), 0·83 d (J = 6·5 Hz), 0·73 d (J = 6·5 Hz); *m/e* 222 (M^+ 100%), 179 (8%), 151 (26%), 138 (24%), 111 (35%). (Found: C, 80·99; H, 11·58. Calc. for $C_{15}H_{26}O$: C, 81·02; H, 11·78%).

Conversion of **8** *to* **9**. To **8** (50 mg) in ether, 1·4 M MeLi (Alfa Inorganics) (10 ml) was added and the soln refluxed 2 hr. Water was added dropwise, then dil HCl; the ether layer was washed with water and dried. The solvent was removed and the residue heated at 90° in formic acid for 1 hr. The reaction mixture was added to water and the suspension extracted with ether which was then washed with water and satd $NaHCO_3$ aq and dried. The solvent was removed and the residue heated with 10% Pd/C (30 mg) at 320° for 2 hr under N_2. Extraction with CH_2Cl_2 and TLC (silica gel, hexane, UV) gave 7 mg of 5-methylcadalene, **9**, R_f 0·5; δ 1·35 d (J = 6 Hz), 2·47 s, 2·58 s, 2·64 s, 7·05 m, 7·6 m; picrate m.p. 100–102° (lit.[21] 102·5–103·5°).

Isomerization of **8** *to* **10** *and* **11**. To a soln of KOH (30 mg) in MeOH (6 ml), was added ketone **8** (36 mg) and the soln refluxed 18 hr. The soln was neutralized with 2N HCl, diluted with water and extracted with CH_2Cl_2. After drying, the solvent was removed and the residue purified by TLC as in the synthesis of **8** to give 18·4 mg of an oil, $[\alpha]_D$ + 55 (*c* 2, CCl_4); δ 2·75 t (J = 4 Hz), 0·85–0·95 (series of overlapping doublets), 0·74 d (J = 6·5 Hz), 0·64 d (J = 6·5 Hz).

Reduction of **2** *with LAH to* **4**. LAH (50 mg) and **2** (from hydrolysis of **1**, 70 mg) were refluxed 1 hr in ether (50 ml). After addition of water, the mixture was extracted with ether, which was washed with water and then with 2N HCl. The acid was cooled, neutralized with dil NaOH aq and extracted with ether. After drying, the solvent was removed to yield 46 mg **4**, oil; δ 5·15 bs, 2·04 [CH_3-16], 1·6 bs, 1·0 s [CH_3-14], 0·95 d (J = 6·5 Hz) and 0·87 d (J = 6·5 Hz) [isopropyl]; *m/e* 235 (M^+ 100%), 204 (-NH_2CH_3 44%), 192 (-isopropyl 47%), 161 (12%), 84 (100%), 71 (31%).

The natural crude formamide fraction (70 mg) was reduced with LAH (1 g) in the same manner to yield 33 mg of a 4:1 mixture of **4** and **15**.

Hofmann elimination of **4** *to* **12**. A soln of **4** (108 mg), KOH (100 mg) and MeI (2 ml) was refluxed 3 hr in EtOH. The mixture was evaporated to dryness and extracted with $CHCl_3$. After removal of the solvent, the residue was triturated with hexane and the insoluble crude quaternary salt, which was not further purified, was dissolved in MeOH (10 mg) containing a little water (0·2 ml) and silver oxide (400 mg) was added. The mixture was stirred 2 hr, then filtered and evaporated to yield 100 mg of quaternary base, which was heated at 165° for 30 min under N_2. Extraction of the mixture with ether and TLC (silica gel, hexane, UV) gave 13 mg of a mixture of hydrocarbons, R_f 0·7. On GLC (3% OV-17, 95° and SE-30, 85°) the retention time of the major component of this mixture coincided exactly with that of an authentic sample of **12**. Repeated TLC of the mixture gave 7 mg of *ca.* 80% **12** (by GLC) $[\alpha]_D$ + 101 (*c* 0·74, CCl_4) [lit.[13] + 120°]; δ 0·95 d (J = 6·5 Hz) [isopropyl], 1·61 bs [allylic methyls], 5·08 b, 5·30 b [olefinic protons].

LAH reduction of **3** *to* **4**. Compound **3** (60 mg) was reduced with LAH by the procedure used for **2**, to give 35 mg **4** identical with **4** derived from **2**.

Ozonolysis of **13**. Ozonized O_2 (Welsbach ozone generator, 0·02 SCFM) was passed for 15 min through a solution of **13** (97 mg) in abs EtOH cooled with a dry ice-acetone bath. The soln was purged with N_2 for 1 min and dimethyl sulfide (1 ml) was added. After standing at room temp. for 18 hr, 2,4 DNPH reagent was added and the ppt filtered. The solid was triturated with CH_2Cl_2 and filtered to yield the insoluble bis 2,4-DNPH derivative of 4-ketopentanal, m.p. 230–233° (lit.[22] 235°). The CH_2Cl_2 soluble fraction was purified by TLC (silica gel, hexane-CH_2Cl_2 1:1) to give the 2,4-DNPH derivatives of acetone and formaldehyde. The filtrate from the 2,4-DNPH reaction was evaporated to dryness and purified by TLC to give the dihydropyrazine derivative of 4-ketopentanal m.p. 148–150° (lit.[22] 153°).

Hydrolysis of **13** *to* **14**. Glacial HOAc (99·7%, 5 ml) was added to **13** (58 mg) and the soln allowed to stand at room temp. 12 hr. The solvent was removed under reduced pressure to give 60·5 mg **14**, ν_{max} 3500, 1680, 1500, 990, 920 cm^{-1}; *m/e* 317 (M^+ 99%), 372 (-NH_2CHO, 100%).

Reduction of **14** *to* **15**. The procedure for the reduction of **1** to **4** was used on **14** (32 mg) and LAH (20 mg) to yield 24 mg **15**. δ 2·2 s [amine methyl]; *m/e* 303 (M^+ 11%), 288 (-CH_3 20%), 272 (-CH_3NH_2 21%), 234 (46%), 166 (100%), 84 (88%).

Reduction of **13** *with lithium-ethylamine to* **17**. The procedure for the reduction of **1** to **5** was followed on **13** (173 mg). TLC (silica gel, hexane, UV) gave 83 mg **17**, R_f 0·5; δ 5·05 b (4 olefinic protons), 2·0 bs (6 allylic CH_2), 1·66 bs, (2 allylic Me), 1·59 s (4 allylic CH_3); *m/e* 274 (M^+).

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REFERENCES

[1] I. Hagedorn and H. Tönjes, *Pharmazie* **12**, 567 (1957); *Chem. Abstr.* **52**, 6362c (1958).
[2] F. Cafieri, E. Fattorusso, S. Magno, C. Santacroce and D. Sica, *Tetrahedron* **29**, 4259 (1973).
[3] L. Minale, R. Riccio and G. Sodano, *Ibid.* **30**, 1341 (1974).
[4] E. Fattorusso, S. Magno, L. Mayol, C. Santacroce and D. Sica, *Ibid.* **30**, 3911 (1974).
[5] B. J. Burreson, C. Christophersen and P. J. Scheuer, *J. Am. Chem. Soc.* **97**, 201 (1975).
[6] B. J. Burreson and P. J. Scheuer, *J. Chem. Soc. Chem. Commun.* 1035 (1974).
[7] W. McFarlane, *J. Chem. Soc. A*, 1660 (1967).
[8] I. D. Kuntz, Jr., P. von R. Schleyer and A. Allerhand, *J. Chem. Phys.* **35**, 1533 (1961).
[9] I. Ugi and F. Bodesheim, *Chem. Ber.* **94**, 1157 (1961).
[10] W. Büchner and R. Dufaux, *Helv. Chim. Acta* **49**, 1145 (1966).
[11] H. O. House and H. W. Thompson, *J. Org. Chem.* **28**, 360 (1963).
[12] L. Westfelt, *Acta Chem. Scand.* **20**, 2852 (1966); B. S. Cheng, Y. H. Kuo and Y. T. Lin, *Chem. Commun.* 565 (1967).
[13] N. H. Andersen, *Tetrahedron Letters* 4651 (1970).
[14] O. Motl, M. Romanuk and V. Herout, *Coll. Czech. Chem. Commun.* **31**, 2025 (1966).
[15] P. S. Kalsi, K. K. Chakravarti and S. C. Bhattacharyya, *Tetrahedron* **19**, 1073 (1963).
[16] A. C. Cope and E. R. Trumbull, *Org. Reactions* **11**, 317 (1969).
[17] R. N. Kniseley, R. P. Hirschmann and V. A. Fassel, *Spectrochim. Acta* **23A**, 109 (1967).
[18] E. Demole and E. Lederer, *Bull. Soc. Chim. Fr.* 1128 (1958).
[19] H. Achenbach and H. Grisebach, *Z. Naturforsch.* **B20**, 137 (1965).
[20] *Dictionary of Organic Compounds* (Edited by J. R. A. Pollock and R. Stevens), Vol. 1, p. 532. Oxford University Press, New York (1965).
[21] S. Dev, *J. Indian Chem. Soc.* **25**, 69 (1948).
[22] A. Mondon, *Angew. Chem.* **64**, 224 (1952).