Tetrahedron. Vol. 28, pp. 4761 to 4767. Pergamon Press 1972. Printed in Great Britain

NEW SESQUITERPENES FROM THE MARINE SPONGE *PLERAPLYSILLA SPINIFERA*

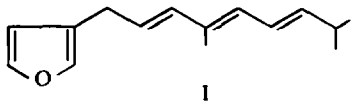
G. CIMINO, S. DE STEFANO, L. MINALE and E. TRIVELLONE

Laboratorio per la Chimica e Fisica di Molecule di Interesse biologico del C.N.R., Arco Felice, Napoli, Italy

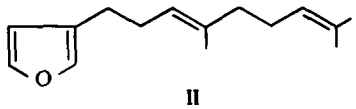
(*Received in the UK 24 February* 1972; *Accepted for publication 9 March* 1972)

Abstract- Two new furanosesquiterpenes, named dehydrodendrolasin (I) and pleraplysillin (IV), have been isolated from the sponge *Pleraplysilla spinifera*. Pleraplysillin represents a new type of monocyclic sesquiterpene skeleton.

OUR STUDIES on the metabolites of various sponges have led to the characterization of a number of furanoterpenes.[1] These have linear C_{25}, C_{30}, or C_{35} chains or truncated (C_{21} or C_{31}) chains. We now describe from the sponge *Pleraplysilla spinifera* two new, rather unstable, furanosesquiterpenes, which are present in very large amounts: one, for which we propose the name of dehydrodendrolasin (*ca* 5% of the dry sponge; I), proved to be related to dendrolasin (II), the odour-substance of the ant *Dendrolasius fuliginosus*[2]; the second, which we called pleraplysillin (*ca* 1·5% of the dry sponge, IV), belongs to a new type of sesquiterpene with an unusual carbon skeleton.



I



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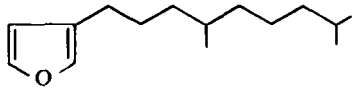


III

Dehydrodendrolasin (I) is a colourless, optically inactive oil, $C_{15}H_{20}O$ (M^+/e 216), with UV absorption typical of a conjugated triene [λ_{max} (C_6H_{12}) 262, 272 and 284 nm; $\log_{10} \varepsilon$ = 4·38, 4·49 and 4·36]. The NMR spectrum (Fig 1a) shows the presence of a β-substituted furan ring (1H broad singlets at δ 7·27 and 7·13 due to furan-α-H's and at δ 6·17 due to a furan-β-H). Thus all the unsaturation in the molecule is

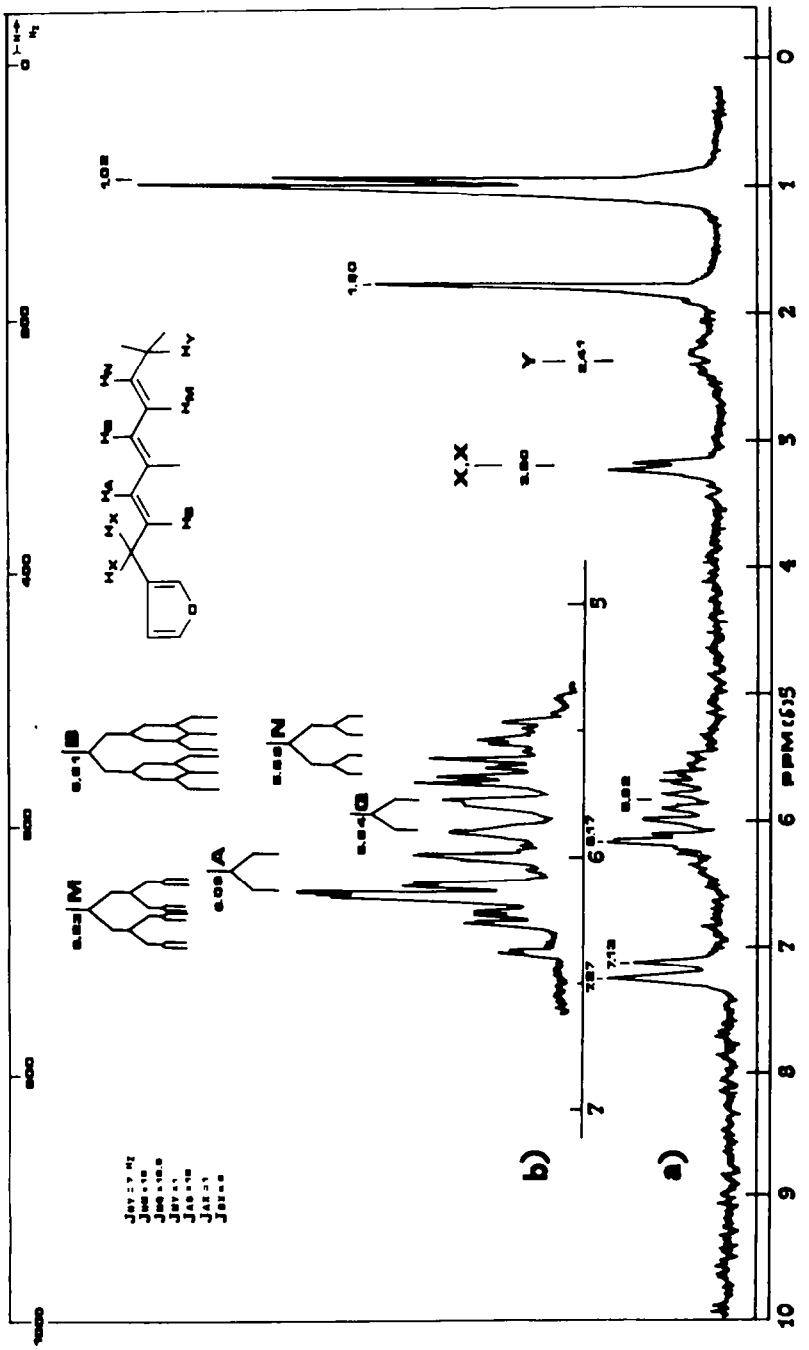
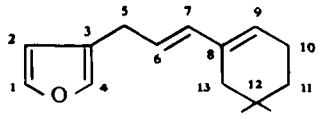


FIG 1a. 100 MHz NMR spectrum (CCl_4) of dehydrodendrolasin (I) recorded at 1000 Hz sweep width; b. Olefinic part of the spectrum recorded at 500 Hz sweep width.

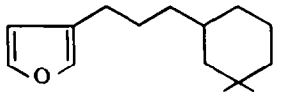
accounted for. The NMR spectrum (Fig 1a) also shows the presence of a vinyl Me group (δ 1·80, bs; 3H), of an isopropyl group on a double bond [1 multiplet at δ 2·41 and 6H doublet (J = 6 Hz) at δ 1·02; irradiation at δ 2·41 causes the 1·02 doublet to collapse to a singlet] and of a methylene group between the furan ring and the conjugated triene system [(δ 3·20, bd, J = 6 Hz; 2H); irradiation on this signal sharpens all three furan protons and produces a modification (see below) of the complex signal spread between δ 5·5–6·35, (overlapped by the broad singlet furan-β-H δ 6·17) which is attributable to five olefinic protons]. Hydrogenation of dehydrodendrolasin (I) gave a decahydroderivative (M^+/e 226), identified as perhydrodendrolasin by direct comparison (MS, NMR and GLC) with an authentic sample. Hydrogenation of I in milder conditions gave a hexahydroderivative (M^+/e 222) characterized as tetrahydrodendrolasin (III) by MS, NMR and IR spectra (Experimental). These results establish the structure I, without stereochemical details, for this new sesquiterpene. The presence in the IR spectrum ($CHCl_3$) of I of a strong band at 962 cm^{-1} (*trans* CH=CH) and the absence of any absorption for *cis* CH=CH in the 730–665 cm^{-1} region suggest a *trans*[3] configuration for the two disubstituted double bonds. This was confirmed by a detailed analysis of decoupling in the NMR spectrum of dehydrodendrolasin which enabled us to make proper assignments of all five olefinic protons as reported in Fig 1b. Irradiation at δ 2·41 (H_Y) collapses the double doublet at δ 5·58 (H_N) to a doublet with J = 15 Hz (J_{NM}), the separation of 7 Hz (J_{NY}) having disappeared, and changes the 8-line signal centered at δ 6·23 (H_M) into a double doublet of 15 (J_{MN}) and 10·5 (J_{MQ}) Hz, the separation of 1·0 Hz (J_{MY}) having disappeared. Irradiation of the vinyl Me protons (δ 1·80) causes a sharpening of the broad doublet at δ 5·84 (H_Q; J_{QM} = 10·5 Hz). The remaining two olefinic protons apparently constitute the AB portion of an ABX_2 system. Irradiation at δ 3·20 [$H_2(X)$] causes a sharpening of the broad doublet centered at δ 6·09 (H_A; J_{AB} = 15 Hz; J_{AX} = 1 Hz; the downfield line of the doublet is overlapped by the signal furan-β-H at δ 6·16) and transforms the double triplet centered at δ 5·61 (H_B) into a doublet with J = 15 Hz (J_{BA}), the separation of 6 Hz (J_{BX}) having disappeared. Both the coupling constant values between H_A and H_B (15 Hz) and between H_M and H_N (15 Hz) require a *trans* interaction, hence the two "end-of-chain" double bonds of the conjugated triene in I must be *trans*. No evidence has been obtained to establish unambiguously the stereochemistry of the central trisubstituted double bond, but the chemical shift of H_A (δ 6·09) suggests that the central bond is *trans*.[4]

In the mass spectrum of dehydrodendrolasin, besides M^+ (*m/e* 215, 90%) there are peaks for M^+-CH_3 (*m/e* 201, 8%), M^+-CH $(CH_3)_2$ (*m/e* 173, 25%), M^+-$CH_2C_4H_3O$ (*m/e* 135, 25%), $C_4H_3OCH_2CH{=}CH^+$ (*m/e* 107, 30%) and $C_4H_3OCH_2^+$ (*m/e* 81, 100%), in agreement with the assigned structure (I).

Pleraplysillin (IV), $C_{15}H_{20}O$ (M^+ 216), is an oily substance, $[\alpha]_D = 0°$, λ_{max} (C_6H_{12}) 226, 234 and 241 (shoulder), $\log_{10} \varepsilon$ = 4·29, 4·31 and 4·11, which gives a colour reaction with the Ehrlich reagent. The NMR spectrum (Fig 2a) shows the presence of a β-substituted furan ring [1H broad singlets at δ 7·25 and 7·13 (furan-α-H's) and at δ 6·17 (furan-β-H)], of two equivalent tert-Me's (6H singlet at δ 6·92) and of three olefinic protons, one of which resonates as a broad doublet (J = 15 Hz) at δ 6·06, the other two appearing as a multiplet at δ 5·56. On hydrogenation over Pd/C, pleraplysillin (IV) gives an Ehrlich-positive tetrahydro derivative (V, M^+ 220), showing peaks at δ 7·25 (1H, bs), 7·13 (1H, bs), 6·17 (1H, bs) and 2·36 (2H, t, J = 6 Hz)

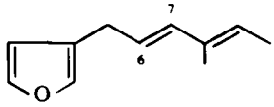


IV



V

(β-methylene-substituted furan ring). This spectral and chemical evidence suggests that pleraplysillin (IV) is a monocyclic sesquiterpene with two double bonds in a 1,3-diene system isolated from the furan ring. The doublet (J = 6 Hz) at δ 3·15 (2H) could be attributed to a methylene between the furan ring and the 1,3 diene system: irradiation (Fig 2c) on this signal sharpens all three furan protons and the two lines of the doublet centered at δ 6·06 (H_A) and transforms the 2H olefinic multiplet at δ 5·56 into a more simple signal from which emerges a clearly visible doublet (H_B: J = 15 Hz) with the characteristic features of the B part of an AB pattern, part A being centered at δ 6·06 (J = 15 Hz); conversely, irradiation on the δ 5·56 multiplet collapses the broad doublet at δ 3·15 to a broad singlet. From this experiment the following partial structure (VI), with the Δ^6 double bond configuration as shown (ν_{max} 960 cm^{-1}) can be deduced.



VI

Oxidative ozonolysis of pleraplysillin gave 3,3[1]-dimethyladipic and malonic acids. This result would point to the structure IV, the alternative 11,11-dimethyl structure being inconsistent with the isoprene rule. Detailed analysis of the 100 M Hz NMR spectrum (Fig 2) of pleraplysillin (IV) with double resonance experiments gives the complete sequence of protons in the cyclohexene ring and completely confirms the proposed structure. The two protons at C-11 appear as a triplet (J = 6 Hz) at δ 1·35 coupled with the C-10 methylene protons resonating as a multiplet at δ 2·15; this latter signal is in turn coupled with the olefinic proton at C-9. Irradiation (Fig 2d) of the multiplet at δ 2·15 (H_2–C–10) collapses the C-11 methylene triplet to a singlet and transforms the olefinic multiplet centered at δ 5·56 into a simpler signal from which emerges a clearly visible broad singlet (H–C–9) overlapping with a system of two triplets with J = 6 (J_{BX}) and J = 15 (J_{BA}) Hz, due to H_B. Conversely, irradiation at the center of the olefinic multiplet (δ 5·56) transforms the C-10 methylene multiplet (δ 2·15) into a triplet with J = 6 Hz, and sharpens the δ 1·88 (2H) broad signal, which must therefore be assigned to methylene at C-13. Finally, irradiation of the methylene

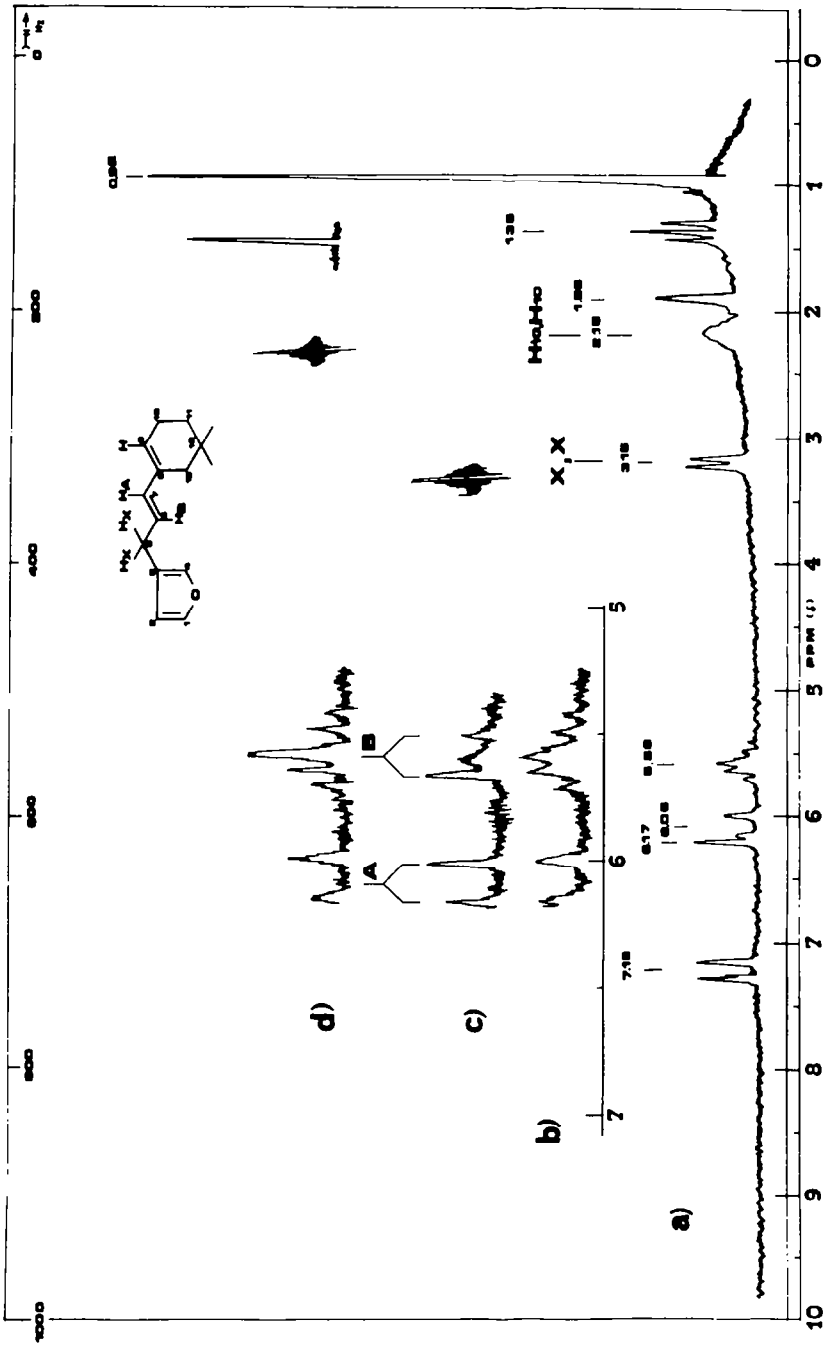
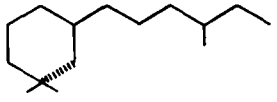


FIG. 2a. 100 MHz NMR spectrum (HCl_4) of pleraplysillin (IV) recorded at 1000 Hz sweep width;
b. Olefinic part of the spectrum recorded at 500 Hz sweep width;
c and d. Decoupling experiments

at C-11 triplet (δ 1·35) causes a transformation of the multiplet at δ 2·15 (H_2–C-10) into a simpler signal with a width at half height of 7 Hz.

In the mass spectrum of pleraplysillin, besides signals for M^+ (*m/e* 216, 100%), M^+-$CH_2C_4H_3O$ (*m/e* 135, 20%), $C_4H_3OCH_2CH{=}CH^+$ (*m/e* 107, 25%) and $C_4H_3OCH_2^+$ (*m/e* 81, 60%), there is a strong peak at *m/e* 160 (30%), corresponding to elimination of isobutene from the dimethylcyclohexene ring by the well-known retro-Diels–Alder process. This further supports the position of the double bond in the cyclohexene ring.

The carbon skeleton of pleraplysillin is so far unique amongst sesquiterpenes since it would seem to arise by a C—C cyclization involving a lateral Me group of



VII

the poly-isoprene chain (VII). Such a cyclization is most unusual, though it occurs also in the 3,3-dimethylcyclohexylidene group (monoterpenes) of boll weevil pheromones.[5] It is unlikely that such cyclization could occur without some prior transformations of the presumed farnesyl precursor, for example by oxygenation of the lateral methyl, which in the case of pleraplysillin is a type of reaction step which must also be involved in the formation of the furanoterpene system.

EXPERIMENTAL

NMR spectra were measured in CCl_4 solns with an HA-100 Varian spectrometer operating at 100 MHz with TMS as internal standard with $\delta = 0$. The u.v. and i.r. spectra were taken on Bausch and Lomb Spectronic 505 and Perkin-Elmer 257 Infracord spectrophotometers. Mass spectra were measured on an AEI MS-9 instrument (70 eV; direct inlet system); the values are given in *m/e* (rel %). GLCs were run using a Carlo-Erba Fractovap model GV with a flame ionization detector; carrier gas N_2, flow rate of 30 ml/min; column length 2 m. Silica gel 0·05–0·2 mm (Merck) was used for column chromatography. Preparative TLC separations were effected using analytical glass packed precoated silica gel plates (20 × 20 cm; F254 Merck). Sponges (*Pleraplysilla spinifera*), collected in the Bay of Naples, were obtained from the supply department of the Zoological Station, Naples.

Extraction and separation. Fresh sponge (13 g dry weight after extraction) was extracted four times with acetone at room temp for 3 days; after concentration *in vacuo*, the aqueous residue was extracted with ether (3 × 100 ml). The combined ethereal extracts were taken to dryness to give 2·5 g of a brown oil. Batches of the crude extract (150 mg) were subjected to column chromatography on SiO_2–$AgNO_3$ (2·5 g $AgNO_3$–15 g SiO_2) to give, by elution with 40–70° light petroleum-benzene (9:1) followed by increasing amounts of benzene, in the following order: pleraplysillin (IV; 12 mg; Found: C, 82·80, H 9·13%. Calc. for $C_{15}H_{20}O$: C, 83·29, H, 9·32%) and dehydrodendrolasin (I; 28 mg; Found: C, 83·50, H, 9·05. Calc for $C_{12}H_{20}O$: C, 83·29, H, 9·32%), as unstable oils. On keeping, they become viscous dark oils. Mixtures of dehydrodendrolasin and the more polar minor components could be separated by TLC on analytical precoated silica gel plates (Merck, 20 × 20 cm) impregnated with $AgNO_3$ (eluent: 40–70 light petroleum–benzene, 8:2) to give an additional quantity (7 mg) of dehydrodendrolasin—UV, IR, NMR and mass spectra are described in the text.

Hydrogenation of dehydrodendrolasin (I)

Perhydrodendrolasin. Dehydrodendrolasin (I; 20 mg) in MeOH (2 ml) was hydrogenated with 2 mg of 10% Pd/C as catalyst at 100° and 120 Atm for 18 hr. Filtn, evap and prep TLC (light petroleum, at 4°) gave mg 11 of perhydrodendrolasin (R_f = 0·9; M^+ 226) identified by direct comparison with an authentic sample (MS, NMR and GLC on 1% SE-30 at 150°).

Tetrahydrodendrolasin (III). Dehydrodendrolasin (20 mg; I) dissolved in MeOH (2 ml) was hydrogenated with 10% Pd/C (2 mg) as catalyst at 80° and 50 Atm for 5 hr. Filn, evap and prep TLC (light petroleum at 4°) gave mg 10 of tetrahydrodendrolasin (oil; III, R_f = 0·7): IR (liquid film) ν_{max} 3140, 1570, 1510, 875 and 780 cm^{-1}; NMR (CCl_4) δ 7·23 (1H, bs, furan-α-H), 7·11 (1H, bs, furan-α-H), 6·14 (1H, bs, furan-β-H), 2·37 (2H, t, J = 6 Hz: $C_4H_3OC\underline{H}_2$), 1·60–1·20 (12H, b signal, $\underline{CH}_2$ and $\underline{CH}$), 0·88 (9H, d, J = 6 Hz, *sec*-Me's); MS 222 (M^+, 25%), 207 (M^+-CH_3, 5%), 179 [M^+-$CH(CH_3)_2$, 10%], 165 [M^+-$CH_2CH(CH_3)_2$, 5%], 151 [M^+-$CH_2CH_2CH(CH_3)_2$, 15%], 137 [$C_4H_3OCH_2CH_2CH_2CH^+(CH_3)$, 10%], 109 ($C_4H_3OCH_2CH_2CH_2^+$, 12%), 95 ($C_4H_3OCH_2CH_2^+$, 95%), 81 ($C_4H_3OCH_2^+$, 100%) and 67 ($C_4H_3O^+$, 10%).

Hydrogenation of pleraplysillin (IV)

Tetrahydroderivative (V). Pleraplysillin (20 mg) in MeOH (2 ml) was hydrogenated with 10% Pd/C as catalyst (2 mg) at 80° and 50 Atm for 5 hr. Filtn, evap and prep TLC (light petroleum at 4°) gave 12 mg of V (R_f = 0·6), as an oil, M^+ 220; NMR (CCl_4) δ 7·25 (1H, bs, furan-α-H), 7·13 (1H, bs, furan-α-H), 6·17 (1H, bs, furan-β-H), 2·36 (2H, t, J = 6 Hz, $C_4H_3OC\underline{H}_2$), 1·8–1·25 (13H, b signal, $\underline{CH}_2$ and $\underline{CH}$) and 0·95 (6H, s, tert-Me's).

Ozonolysis of pleraplysillin (IV). Pleraplysillin (30 mg) in EtOAc (5 ml) was ozonized (2% O_3) for 3 hr at −15°. After evap of solvent *in vacuo*, the ozonide was decomposed with water containing a few drops of H_2O_2. The mixture was extracted continuously for 5 hr with ether. The extract was concentrated and treated with CH_2N_2. After removal of solvent, the degradation products were analysed by GLC (2·5% SE-30 and 10% DEGS at 120° and 190°, respectively) and found to comprise methyl malonate and methyl 3,3-dimethyladipate by comparison with authentic samples.

Acknowledgments—The authors thank Professor F. Piozzi (University of Palermo, Italy) for a sample of perhydrodendrolasin and Professor M. Sarà (University of Genova) for identifying the sponge. Thanks are also due to Mr. A. Milone for mass measurements and to Mr. G. Scognamiglio for technical assistance.

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