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POLYPRENYL DERIVATIVES FROM THE SPONGE *IRCINIA SPINOSULA*

2-POLYPRENYLBENZOQUINONES, 2-POLYPRENYLBENZOQUINOLS, PRENYLATED FURANS AND A C-31 DIFURANOTERPENE

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

Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico del C.N.R., Arco Felice, Naples, Italy

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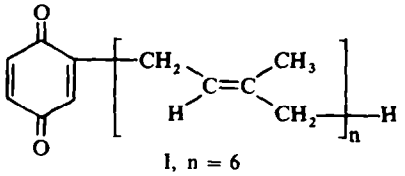
Abstract—Unsubstituted prenylated benzoquinones, a novel group of terpenoid quinones, the corresponding quinols, together with the hydroxylated 2-octaprenyl-quinol (VII) have been identified in the marine sponge *Ircinia spinosula*. From the same sponge we have also isolated the prenylated furan derivatives XI, XII and XIII, named furospinosulin-1, -2 and -3, and an unusual C-31 difuranoterpene, difurospinosulin (XIV).

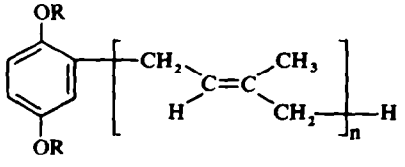
The synthesis of 2-heptaprenyl-1,4-benzoquinol (V) is reported.

TERPENOID QUINONES such as vitamins K (naphthoquinones), tocopherol quinones, ubiquinones and plastoquinones (all benzoquinones) are widespread in nature and it seems that they play an important role in photosynthesis and electron transport.[1]

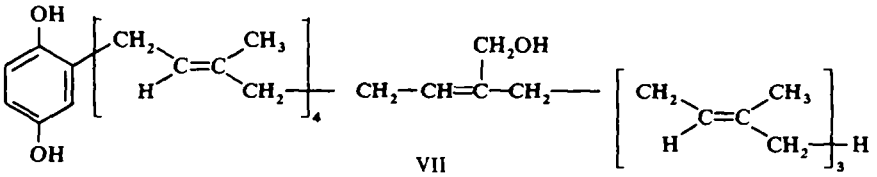
In this paper we report the occurrence in the marine sponge, *Ircinia spinosula*, of the 2-polyprenyl-1,4-benzoquinones I, II and III, the corresponding quinols IV, V and VI present in the solvent extracts in much larger quantities, together with the hydroxylated 2-octaprenylquinol VII and minor quantities of the prenylated furan derivatives XI, XII and XIII and the C-31 difuranoterpene XIV.



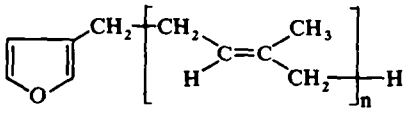
I, n = 6
II, n = 7
III, n = 8



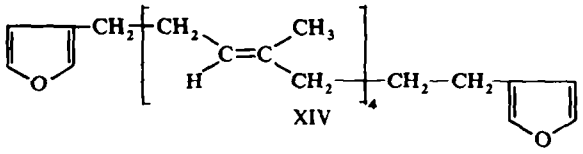
IV, n = 6 R = H VIII, n = 6 R = CH_3CO—
V, n = 7 R = H IX, n = 7 R = CH_3CO—
VI, n = 8 R = H X, n = 8 R = CH_3CO—



VII



XI, n = 4
XII, n = 5
XIII, n = 6



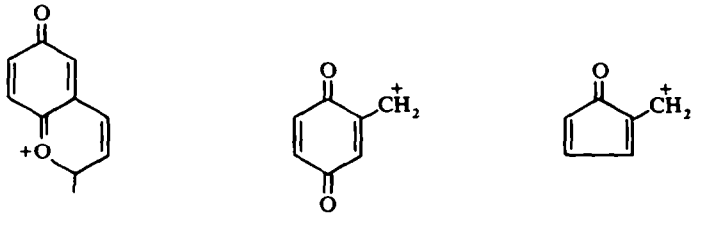
XIV

Unsubstituted prenylated benzoquinones have not been previously isolated from natural sources apart from the occurrence of γ,γ-dimethylallyl-1,4-benzoquinone in *Phagnalon saxitale*[2] and a postulated 2-polyprenyl-1,4-benzoquinone in *Ps. ovalis*.[3]

Two different samples of the sponge *Ircinia spinosula*, collected in the Bay of Naples in October 1970 (sample 1) and in February 1971 (sample 2), were analyzed.

Sample 2: Fresh material was extracted with MeOH and subsequently with acetone: the crude extract was chromatographed on a silica gel column to give, in order of polarity, *trans* squalene, a mixture of the quinones I, II and III (fraction a), a mixture of quinols IV, V and VI (fraction b) and the hydroxylated 2-octaprenylquinol VII.

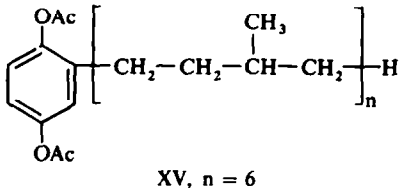
Fraction (a): The IR spectrum (ν_{max} 1660 and 1600 cm^{-1}) is consistent with a *p*-benzoquinone structure, and furthermore, the UV spectrum, which shows an intense band at 245 nm, a medium band at 315 nm and a much weaker absorption in the visible region at 440 nm, is almost identical with that of γ,γ-dimethylallyl-1,4-benzoquinone.[2] The NMR spectrum shows three quinonoid protons (2H broad singlet at δ 6·64 and 1H broad singlet at 6·43). The mass spectrum shows molecular ion peaks at *m/e* 652 (M^+ of III), 584 (M^+ of II) and 516 (M^+ of I, very small) and a series of peaks at M^+–69–(68)n arising from successive loss of isoprene units, while the base peak at *m/e* 161 corresponds to the ion (*a*) characteristic of prenylated-1,4-benzoquinones.[4]



(a) (b) (c)

This suggests that fraction (a) is a mixture of three 2-polyprenyl-1,4-benzoquinones I, II and III. The NMR spectrum (Experimental) provides confirmation of the isoprenoid structure and suggests the *all trans* configuration. Further evidence is given below.

Fraction (b): The UV (λ_{max} 294), IR (ν_{max} 3350, 1500, 1445, 910, 785 and 730 cm^{-1}) and NMR (3H broad singlet at δ 6·46) spectra are consistent with a monosubstituted-1,4-dihydroxybenzene structure, supported by the conversion, on oxidation with Ag_2O, to a mixture of quinones with all spectral features (UV, IR, NMR and MS) identical with those of fraction (a) (mixture of I, II and III). The mass spectrum of mixture (b) shows, as expected, the molecular ions at *m/e* 654 (M^+ of VI), 586 (M^+ of V) and 518 (very small, M^+ of IV), and a fragmentation pattern (M^+–69–(68)n) consistent with the presence of polyprenyl chains. The base peak at *m/e* 123 corresponds to the ion $C_6H_3(OH)_2CH_2^+$, while the prominent peaks at *m/e* 161 (*a*), 121 (*b*), 95 (123–CO) and 93 (*c*) confirm structures IV–VI. On acetylation fraction (b) gave a mixture of diacetates [*m/e* 738 (M^+ of X), 670 (M^+ of IX) and 602 (M^+ of VIII)] separable by GLC into three clear components, the middle peak having the largest area on the chromatogram (Table 1). A straight line resulted when the logarithm of their retention times was plotted against the number of isoprene units (Fig 1) which is good evidence that the original mixture of prenylated-quinols and -quinones contained three components containing respectively six, seven and eight isoprene residues all in *trans* configuration.* A parallel straight line plot could also be derived from the perhydro-acetates [*m/e* 754 (M^+ of XVII), 684 (M^+ of XVI) and 614 (M^+ of XV)] (Fig 1).



XV, n = 6
XVI, n = 7
XVII, n = 8

Finally ozonolysis of the original mixture of phenols yielded acetone, malonic and levulinic acids.

The structure of the quinol having a C_{35} side chain V has been confirmed by synthesis. Hydroquinone was condensed with farnesyl-geranyl-linalool (*trans*) in the presence of BF_3 etherate[2] to give 2-heptaprenylquinol (V) which was spectroscopically identical, where relevant, with the natural mixture of prenylated quinols. Its diacetate had a retention time on GLC identical with that of the middle component of the mixture of acetates derived from the natural quinols.

Hydroxylated 2-octaprenyl-1,4-dihydroxybenzene (VII)

The more polar component from the second sample of *Ircinia spinosula* has

* It is known that prenylated derivatives differing not only in the number of isoprenoid units, but also in the *cis–trans* nature of these units, give broadened GLC peaks and log. retention time/isoprene number plots which do not fall perfectly on a straight line.[5]

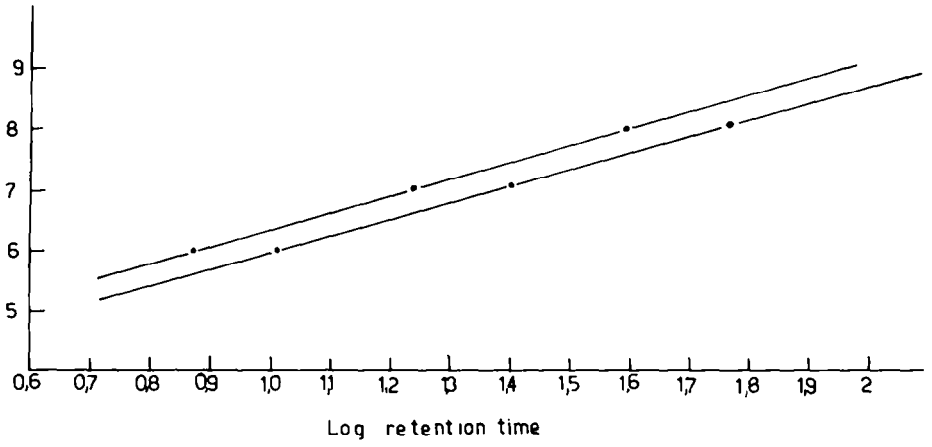


FIG 1. Relationship between $\log_{10}$ retention time at 310° and the number of isoprene residues per molecule of each component of the mixture of prenylated quinol acetates (lower line) and the number of saturated isoprene residues per molecule of each component of the mixture of perhydroprenyl quinol acetates (upper line). Details of GLCs are given in Table 1

molecular formula $C_{46}H_{70}O_3$ (mass spectrum and elemental analysis). The UV (λ_{max} 292 nm, ε 3100) and NMR multiplet δ 6·54 (3H) suggest a monosubstituted-1,4-dihydroxybenzene structure and, on oxidation with Ag_2O, it was converted into a benzoquinone (λ_{max} 249, 315 and 440 nm; ν_{max} 1660 and 1600 cm^{-1}) which showed HO absorption at 3600–3250 cm^{-1}. A prominent peak at M-18 in the mass spectra of both quinol and quinone confirms the presence of an alcoholic OH group, which appears to be primary and allylic (2H singlets at δ 4·11 and 4·02 in the spectra of quinol and quinone, respectively). Acetylation of VII gave a triacetate (M^+ 796, ν_{max} 1770 and 1735 cm^{-1}), the NMR spectrum of which showed a downfield shift (0·5 ppm) of the —CH_2OAc signal relative to that of the parent compound. Both NMR and mass spectra (Experimental) suggest a hydroxylated octaprenyl structure which was established by hydrogenation of the triacetate to give the perhydro-diacetate (XVII)-hydrogenolysis of the allylic acetyl group occurring simultaneously. On GLC it had a retention time identical with that of the last component of the mixture of the perhydroprenyl acetates derived from I, II and III.

From the mass [prominent peaks at *m/e* 583 (M^+–18–69) and at *m/e* 161 (ion *a*)] and NMR (3H singlets at δ 1·73, Me of the first isoprene unit and 1·67, *cis*-Me of the terminal isoprene unit) spectra, it was decided that neither the terminal nor the first isoprene unit contained the additional OH group. Furthermore, the mass spectrum of the triacetate (the central part of the mass spectra of both quinol and quinone are confusing) suggests that the OH must be located in the *fifth* isoprene unit counting from the quinone ring. In fact the mass spectrum shows peaks at *m/e* M^+–69–$(68)_n$ (n = 0 to 2), arising from successive loss of three isoprene units; further peaks are found at *m/e* 465 which derive from M^+–69–$(68)_2$ (*m/e* 591) by loss of the acetylated isoprene unit, and at *m/e* 465–$(68)_n$.

Sample 1. Extraction of fresh material as before and subsequent chromatography gave a less polar fraction (c), a mixture of the quinones I and II, (fraction d), and in

very large quantities a mixture of quinols IV and V (fraction e: 10 g from 514 g of dry sponge). Further chromatography of fraction (c) afforded *trans*-squalene and the prenylated furans XI (trace), XII (40 mg from 514 g of dry sponge) and XIII (100 mg from 514 g of dry sponge) named furospinosulin-1, -2, and -3, respectively, and the difuranoterpene XIV (13 mg from 514 g of dry sponge) designated as difurospinosulin.

Prenylated furans: Furospinosulin-1 (XI), -2 (XII) and -3 (XIII) were shown to be members of one isoprenologous series, as they defined a straight line when the logarithm of the retention time was plotted against isoprene unit number (Fig 2): they resembled higher isoprenologues of perillene (C-10), isolated from the leaves of *Perilla citriodora*[6] and dendrolosin (C-15), isolated from the ant *Dendrolosius fuliginosus*.[7] Here we present the evidence supporting the structure of furospinosulin-3 (XIII), the most abundant component of this series, the evidence for the structures XI and XII assigned to furospinosulin-1 and -2 being the same (Experimental).

Furospinosulin-3, (XIII), $C_{35}H_{54}O$ (mass spectrum and elemental analysis), is a colourless, optically inactive oil. The NMR spectrum indicates the presence of a β-methylene-substituted furan ring [two broad singlets at δ 7·22 (1H) and 7·10 (1H), attributable to two α-hydrogens, a broad singlet at δ 6·16 due to one β-hydrogen, and one 2H triplet at δ 2·34 (J = 6 Hz), assignable to a methylene group attached to the furan ring], and of an *all-trans* hexaprenyl side chain [a 6H broad multiplet at δ 5·09 (CH=C), a 22H broad singlet at δ 1·98 (CH_2—C=C), a 3H singlet at δ 1·66 (*cis* Me of the terminal isoprene residue) and a 18H singlet at δ 1·58 (*trans* Me—C=C)]. The UV (λ_{max} 218 nm) and IR (ν_{max} 3140, 1570, 1500, 1165, 1065, 1020, 875, 780 cm^{-1})[8,9] spectra and positive Ehrlich test confirm the presence of a furan ring.

Mass spectrometry further supports the presence of a furan ring [ions at *m/e* 81 ($C_4H_3OCH_2^+$) and 67 ($C_4H_3O^+$)] and shows that the polyprenyl side chain contains six isoprene units: the spectrum, in fact, shows peaks at M^+ (*m/e* 490), M^+–15 (loss of Me), M^+–69 (loss of terminal isoprene unit), M^+–69–$(68)_n$, where n = 1–5 (sequential loss of five internal isoprene units). Thus furospinosulin-3 must have structure XIII. On ozonolysis it yielded acetone, succinic and levulinic acids.

Difurospinosulin (XIV), $C_{31}H_{44}O_2$ (mass spectrum and elemental analysis) is an oily Ehrlich-positive substance with absorption at 218 nm (furan ring chromophore). The NMR spectrum reveals two β-methylene-substituted furan rings (Experimental) and suggests the presence of the *all-trans*-tetraprenyl [CH_2—CH=C(CH_3)—CH_2]$_4$ chain (4H broad multiplet at δ 5·05 (CH=C), 16H broad singlet at δ 2·00 (CH_2—C=C) and a 12H singlet at δ 1·58 (*trans* Me—C=C)]. Moreover, a broad multiplet at δ 1·64 (2H, —CH_2—$\underline{CH_2}CH_2$—) and prominent peaks in the mass spectrum at *m/e* 163 ($C_4H_3OCH_2CH_2CH_2C(CH_3)$=CH—$CH_2^+$) and at *m/e* 149 ($C_4H_3OCH_2CH_2CH$=C($CH_3$)—$CH_2^+$) suggest that difurospinosulin has structure XIV. Conclusive proof was obtained by oxidative ozonolysis, which gave succinic, levulinic and 5-oxohexanoic acids.

The co-occurrence of the C-35 linear furanoterpene XIII and the C-31 linear difuranoterpene XIV in the same organism strongly suggests that the latter is derived, biogenetically, from the former by loss of four carbon atoms and terminal furan ring closure. This further supports the biogenetic hypothesis that the C-21 furanoterpenes occurring in other sponge species (*Spongia nitens*, *S. officinalis* and *Hyppospongia communis*) are degraded sesterterpenes.[9] Moreover it is noteworthy that recently we have isolated from the sponge *Ircinia oros* two linear sesterterpenes.[10]

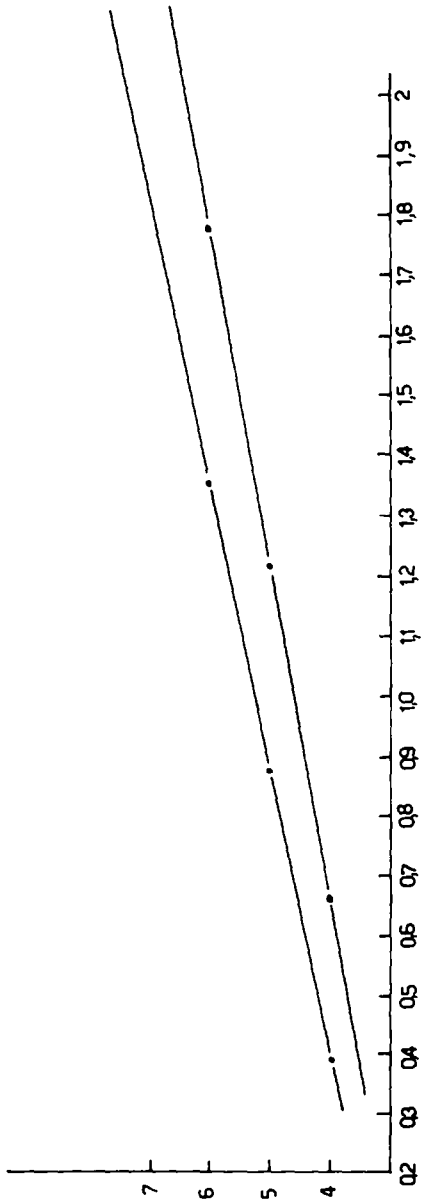


FIG 2. Relationship between $\log_{10}$ retention time at 250° (lower line) and 270° (upper line) and the number of isoprene residues per molecule of prenylated furan derivatives XI, XII and XIII. Details of GLCs are reported in Table 1

The study of the biological role of prenylated 1,4-benzoquinones in sponges, and the presence or absence of ubiquinones,[11] requires further study.

EXPERIMENTAL

NMR spectra were determined on a Varian HA-100 spectrometer operating at 100 MHz with TMS as internal standard with $\delta = 0$; mass spectra were determined on an AIE MS-9 mass spectrometer. UV and IR spectra were taken on Bausch and Lomb Spectronic 505 and Perkin–Elmer 257 Infracord spectrophotometers. Columns chromatography was carried out on a silica gel 0·05–0·2 mm (Merck), TLC and PLC were carried out on precoated silica gel plates (Merck). Analyses were performed by the microanalytical service of our laboratory under the direction of Mr. S. De Rosa.

Extraction of Ircinia spinosula (*sample 2*). Fresh material (75 g, dry after extraction) was extracted (× 3) with MeOH at room temp for 3 days. The combined extracts (1 l) were concentrated and the remaining aqueous soln was extracted with ether: the solvent taken to dryness to give an oily residue (1·5 g). The sponge was then digested with cold acetone (0·5 l) for 1 day, filtered and the extract taken to dryness. The oily residue (1·4 g) was combined with the residue of the MeOH extraction (1·5 g) and chromatographed on a SiO_2 (130 g) column (ϕ 3 cm). Fractions of 100 ml were collected. Elution with C_6H_6 gave *all-trans* squalene (30 mg) in the first two fractions which was rechromatographed in light petroleum on silica gel and identified by comparison (MS, NMR, GLC) with an authentic specimen, and mixture (a) (I, II and III: 110 mg) in the successive fractions (fractions 15–30): λ_{max} (C_6H_{12}) 245, 315 and 440 nm, $E^{1\%}_{1cm}$ 270, 27, 0·78: ν_{max} (liquid film) 1660 and 1600 cm^{-1}: *m/e* (%) 652 (2, M^+ of III) 584 (10, M^+ of II, major component) and 516 (5, M^+ of I) 515 (15, 584–69), 447 (5, 516–69 and 515–68), 379 (5, 447–68): 311 (41, 379–68), 243 (3, 311–68), 175 (4, 243–68), 161 (100, ion *a*), 123 [$C_6H_3(OH)_2$—CH_2^+], 121 (18, *b*), 81 (30) and 69 (70; NMR (CCl_4) 6·64 (2H, bs, ring protons), 6·43 (1H, bs, ring proton), 5·31 (1H, t, J = 6 Hz, Q—CH_2—C<u>H</u>=C), 5·08 (bm, CH=C), 3·08 (2H, d, J 6 Hz, Q—C<u>H</u>$_2$—CH=C), 2·05 and 1·98 (each b singlet, CH_2—C=C), 1·64 (6H, s, *cis* Me of the terminal isoprene residue and *trans* Me of the first isoprene unit) and 1·58 (s, CH_3—C=C *trans*).[12] Further elution with C_6H_6 ether (9:1), gave 335 mg of mixture (b) (IV, V and VI) (fractions 40–44): λ_{max} (MeOH) 294 nm, $E^{1\%}_{1cm}$ 53: ν_{max} 3350, 1500, 1445, 910, 785 and 730 cm^{-1}: δ (CCl_4) 6·46 (3H, m, ArH) 5·25 (1H, t, J = 6 Hz, Ar-CH_2—C<u>H</u>=C) 5·07 (bm, CH=C) 3·21 (2H, d, J = 6 Hz, Ar-C<u>H</u>$_2$—CH=C), 1·98 (bs, CH_2C=C) 1·72 [3H, s, Ar-CH_2CH=C(C<u>H</u>$_3$) (*trans*)] 1·66 (3H, s, *cis* Me of the terminal isoprene residue), 1·57 (s, *trans* Me—C=C): *m/e* (%) 654 (2, M^+ of III), 586 (8, M^+ of II), 518 (4, M^+ of I) 517 (4, M^+ of II–69), 449 (4, 517–68 and 518–69), 381 (4, 449–68), 313 (5, 381–68), 245 (8, 313–68), 177 (20, 245–68), 163 [32, $C_6H_3(OH)_2$—CH_2—CH=$C^+(CH_3)$], 161 (30, *a*), 123 (100; $C_6H_3(OH)_2$—$\overset{+}{C}H_2$), 121 (35, *b*) 95 (48, 123–CO), 93 (35, *c*), 81 (40) and 69 (80), and alcohol VIII (198 mg) (fractions 52–58); λ_{max} (C_6H_{12}) 292 nm, ε 3, 100; ν_{max} (film) 3300 (b), 1500, 1445, 800 and 760 cm^{-1}; δ (CCl_4) 6·54 (3H, bs, ArH), 5·29 (1H, t, J = 6 Hz, $ArCH_2C$<u>H</u>=C), 5·09 (m, CH=C), 4·11 (2H, s, —C<u>H</u>$_2$—OH), 3·26 (2H, d, J = 6 Hz, ArC<u>H</u>$_2$—CH=C), 2·04 (m, CH_2—C=C) 1·73 (3H, s, *trans* Me of the first isoprene unit), 1·67 (3H, s, *cis* Me of the terminal isoprene residue), 1·58 (s, *trans* Me—C=C); *m/e* (%) 670 (3, M^-), 668 ($2M^+$ —2H), 652 (4, M^+—H_2O), 583 (3, M^+—H_2O–69), 515 (5, M^+—H_2O–69–68), 163 (15, Ar-CH_2—CH=$\overset{+}{C}$—CH_3), 161 (40, ion *a*) 123 (60, $C_6H_3(OH)_2$—$\overset{+}{C}H_2$) 121 (30, *b*), 95 (35, 123–CO), 93 (30, *c*), 81 (75), 69 (100). (Found: C, 81·70; H, 10·15. $C_{46}H_{70}O_3$ requires C, 82·39: H, 10·45%).

Extraction of Ircinia spinosula (*sample 1*). Fresh material (514 g, dry after extraction) was extracted with MeOH and subsequently with acetone. Working as up above gave 30 g of a syrup which was chromatographed in C_6H_6 over silica gel (800 g). The column was eluted with 20 fractions (200 ml each) of C_6H_6 and 40 fractions of C_6H_6–ether (9/1). Fractions 2–3 yielded an oil (0·4 g) (see below). Fractions 19–25 yielded mixture *c* (quinones I and II) (0·55 g): λ_{max} (C_6H_{12}) 245, 315, 440 nm, $E^{1\%}_{1cm}$ 300, 30, 0, 8: ν_{max} (film) 1660 and 1600 cm^{-1}: *m/e* (%) 584 (2, M^+ of II), 516 (10, M^+ of I), 515 (0·5, 584–69), 447 (3, 516–69 and 515–68), 379 (3, 447–68), 311 (2, 379–68), 243 (3, 311–68), 175 (6, 243–68), 161 (100; ion *a*), 123 (15; $C_6H_3(OH)_2$—CH_2^+), 121 (15, *b*), 81 (30), and 69 (65): NMR (CCl_4) δ 6·64 (2H, bs, ring protons), 6·43 (1H, bs, ring proton), 5·31 (1H, t, J = 6 Hz, Q—CH_2—C<u>H</u>=C), 5·08 (bm, —CH=C), 3·08 (2H, d, J = 6 Hz, Q—C<u>H</u>$_2$—CH=C), 2·05 and 1·98 (each *b* singlet, —CH_2—C=C), 1·64 (6H, s, *cis* Me of the terminal isoprene residue and *trans* Me of the first isoprene unit),[5] 1·58 (s, *trans* Me—C=C). Franctions 27–33 yielded mixture (*d*) (quinols IV and V) 10 g: M^+ 586 (IV) and 518 (V; major component): λ_{max} (MeOH) 294 nm, $E^{1\%}_{1cm}$ 55: The mass, IR, and NMR spectra are identical with those of the mixture of 2-polyprenyl-1,4-benzoquinols IV, V, and VI isolated from sample 2 of *Ircinia spinosula*.

The less polar material (0·4 g) left by fractions 2–3 was rechromatographed over silica gel (7 g) using light petroleum (40–70°) as eluent. Fractions of 5 ml were collected and monitored by TLC. Fractions 4–9 yielded *all-trans* squalene (55 mg) identified by comparison (MS, NMR, GLC and TLC) with an authentic specimen. Fractions 10–13 yielded XI (10 mg: oil): λ_{max} (C_6H_{12}) 218 nm, ε 4500; *m/e* (%) 354 (5, M^+), 339 (2, M^+-15), 285 (5, M^+-69), 217 (4, $M^+-69-68$), 149 (7, $M^+-69-68-68$), 81 (60, $C_4H_3OCH_2^+$), 69 (100), and 67 (15, $C_4H_3O^+$): NMR (CCl_4) δ 7·22 (1H, bs, furan α-H), 7·10 (1H, bs, furan α-H), 6·16 (1H, bs, furan β-H), 5·09 (4H, bm, CH=C), 2·34 (2H, t, J = 6 Hz, $C_4H_3O-C\underline{H}_2-CH_2$), 1·98 (14H, bs, $-CH_2-$), 1·66 (3H, s, *cis* Me of the terminal isoprene residue) and 1·58 (12H, s, *trans* Me—C=C). (Found: C, 84·25; H, 10·50. $C_{25}H_{38}O$ requires C, 84·69; H, 10·80%). Fractions 14–23 yielded XII (40 mg: oil): λ_{max} (C_6H_{12}) 218 nm, ε 4700: *m/e* (%) 422 (5, M^+), 353 (5, M^+-69), 285 (5, $M^+-69-68$), 217 (6, $M^+-69-68-68$), 149 (12, $M^+-69-68-68-68$), 81 (85, $C_4H_3OCH_2^+$), 69 (100), and 67 (14, $C_4H_3O^+$): δ (CCl_4) 7·22 (1H, bs, furan α-H), 7·10 (1H, bs, furan α-H), 6·16 (1H, bs, furan β-H), 5·09 (5H, bm, CH=C), 2·34 (2H, t, J = 6 Hz, $C_4H_3O\underline{CH}_2-CH_2$), 1·98 (18H, bs, $-CH_2-$), 1·66 (3H, s, *cis* Me of the terminal isoprene residue) and 1·58 (15H, s, *trans* Me—C=C). (Found: C, 84·90; H, 10·62. $C_{30}H_{46}O$ requires C, 85·25; H, 10·97%). Fractions 26–55 yielded XIII (100 mg: oil) λ_{max} (C_6H_{12}) 218 ε 4800: *m/e* (%) 490 (30, M^+), 475 (3, M^+-15), 421 (15, M^+-69), 353 (15, $M^+-69-68$), 285 (15, $M^+-69-68-68$), 217 (10, $M^+-69-68-68-68$), 149 (20, $M^+-69-68-68-68-68$), 81 (70, $C_4H_3O\overset{+}{C}H_2$), 69 (100) and 67 (20, $C_4H_3O^+$): δ (CCl_4) 7·22 (1H, bs, furan α-H), 7·10 (1H, bs, furan α-H), 6·16 (1H, bs, furan β-H), 5·09 (6H, bm, CH=C), 2·34 (2H, t, J = 6 Hz, $C_4H_3O\underline{CH}_2-CH_2$), 1·98 (22H, bs, CH_2), 1·66 (3H, *cis* Me of the terminal isoprene residue) and 1·58 (18H, s, *trans* Me—C=C). (Found: C, 85·96; H, 11·2. $C_{35}H_{54}O$ requires C, 85·71; H, 11·02%).

Finally fractions 58–68 yielded XIV (13 mg, oil): λ_{max} (C_6H_{12}) 218 nm, ε 8900: *m/e* (%) 448 (20, M^+), 433 (10, M^+-15), 163 (35, $C_4H_3OCH_2CH_2\overset{+}{C}H-CH(CH_3)=CH_2$), 149 (20, $C_4H_3OCH_2CH_2CH=C(CH_3)-\overset{+}{C}H_2$), 135 (50, $C_4H_3OCH_2CH_2CH=\overset{+}{C}-CH_3$), 95 (35, $C_4H_3OCH_2CH_2^+$), 81 (100, $C_4H_3OCH_2^+$), and 67 (20, $C_4H_3O^+$): δ (CCl_4) 7·24 (2H, bs, furan α-H), 7·11 (2H, bs, furan α-H), 6·16 (2H, bs, furan β-H), 5·05 (4H, bm, CH=C), 2·34 (4H, bt, J = 6 Hz, $C_4H_3O-\underline{CH}_2-CH_2$), 2·00 (16H, bs, $CH_2-C=C$), 1·64 (2H, bm, $CH_2-C\underline{H}_2-CH_2$) and 1·58 (12H, s. Me—C=C *trans*). (Found: C, 82·80; H, 9·50. $C_{31}H_{44}O_2$ requires C, 83·04: H, 9·82%).

Acetlyation and hydrogenation of the prenylated quinol mixtures and of the alcohol VII. Acetates were prepared by mixing the appropriate phenols (100–200 mg) with Ac_2O (2–4 ml) and pyridine (3–6 drops) and refluxing for 30 min. Ice water (10–20 ml) was then added and the products were extracted with ether and purified by silica gel chromatography in C_6H_6.

Acetates of the prenylated 1,4-benzoquinol mixtures: λ_{max} (C_6H_{12}) 266 nm ($E^{1\%}_{1cm}$ 106); ν_{max} (film) 1770, 1205, and 1170 cm^{-1}; δ (CCl_4)—CH_3CO 2·18 (s) and 2·16 (s) integrating together for 6H; *m/e* 738 (M^+ of X), 670 (M^+ of IX, major component), and 602 (M^+ of VIII) for the mixture from sample 2 of the sponge, and 670 (M^+ of IX) and 602 (M^+ of VIII, major component) for the mixture from sample 1. GLC data are reported in Table 1.

TABLE 1. GAS CHROMATOGRAPHIC DATA FOR THE MIXTURE OF POLYPRENYL-1,4-BENZOQUINOL ACETATES AND THE CORRESPONDING PERHYDRO DERIVATIVES AT 310°

Component	Retention time (min)	Peak area (% of total)	
		Sample 1	Sample 2
VIII	7·09	66·11	33·03
IX	17·01	33·89	62·04
X	40·16	—	4·92
X	5·87		
XVI	17·60		
XVII	30·95		

GLCs (C. Erba GV apparatus) were carried out on silanized chromosorb W coated with SE-30 (1% W/W) and packed in a 2 m long silanized glass tube with an internal diameter of 3 mm. Carrier gas flowed at 37 ml/min.

Acetate of VII: M^+ 796; λ_{max} (C_6H_{12}) 268 nm, ε 1950; ν_{max} (film) 1770, 1735, 1235, 1025, 1205 and 1170 cm^{-1}. δ CH_3CO—($CDCl_3$) 2·26 (bs) and 2·25 (s) integrating together for 9H, δ $C\underline{H}_2$ OAc 4·60 (2H, s): *m/e* (%), 796 (2, M^+), 736 (17, M^+–60), 727 (2, M^+–69), 667 (4, M^+–60–69), 659 (2, M^+–69–68), 599 (5, M^+–60–69–68), 591 (2, M^+–69–68–68), 531 (6, M^+–60–69–68–68), 465 (12, M^+–69–68–68—CH_2—CH=C(CH_2OAc)—CH_2), 397 (18, 465–68), 329 (4, 397–68), 241 (5, 329–68), 81 (90), 69 (100). (Found: C, 78·55: H, 9·70: $C_{52}H_{76}O_6$ requires C, 78·35: H, 9·61%).

Portions of these acetates were hydrogenated for 5 hr at room temp and pressure using Pd/C (10%) as catalyst and C_6H_{12} as solvent. The mixture of prenyl-1,4-benzoquinol acetates gave the corresponding perhydroprenyl derivatives: δ (CCl_4) 6·89 (3H, bs, ArH), 2·27 (2H, t, J = 6 Hz, $ArCH_2$—) 2·22 and 2·20 (each s, intergrating together for 6H, CH_3CO—) 1·23 (bs, CH_2 and CH), 0·85 (complex signal Me): M^+ 754 (M^+ of XVII), 684 (M^+ of XVI, major component) and 614 (M^+ of XV) for the mixture from sponge sample 2, and 684 (M^+ of XVI) and 614 (M^+ of XV, major component) for sample 1. GLC data of the perhydrophenyl-1,4-benzoquinol acetates are reported in Table 1.

The hydrogenation product from the acetate of VII was the perhydroprenyl derivative XVII: M^+ 754, λ_{max} (MeOH), 267 nm, ε1·800: δ (CCl_4) 6·89 (3H, bs, ArH), 2·27 (2H, t, J = 6 Hz, Ar-CH_2), 2·22 and 2·20 (each s, integrating together for 6H, CH_3CO—), 1·23 (bm, CH_2 and CH), 0·85 (complex signal Me). Retention time in GLC (for experimental conditions see Table 1) was 30·95 min identical with that of XVII.

Ozonolysis of the mixtures of polyprenyl-1,4-benzoquinols. 300 mg of mixtures of polyprenyl-1,4-benzoquinols (isolated from each sponge sample) in EtOAc (50 ml) was ozonized (2% O_3) for 3 hr at −15°. After evaporation of solvent *in vacuo*, the ozonide was decomposed with H_2O containing a few drops of H_2O_2 by heating for 30 min on a steam-bath, and steam-distilled into 200 ml of 2N HCl saturated with 2,4-dinitrophenylhydrazine. The yellow precipitate was collected and purified by PLC in C_6H_6 to give acetone dinitrophenylhydrazone, m.p. 124–125·5° (from EtOH) (50 mg), identical (m.m.p., MS, and NMR) with an authentic sample. The residue left after steam-distillation 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 analyzed by GLC (5% SE-30 and 10% DEGS at 100° and 175°, respectively) and found to comprise dimethyl malonate and methyl levulinate, by comparison with authentic samples.

Synthesis of 2-heptaprenyl-1,4-dihydroxybenzene (V). To a soln of *all-trans* farnesyl-geranyl-linalool (490 mg) in dry dioxan (1 ml), hydroquinone (220 mg) in dry dioxan (1 ml) and BF_3–etherate (0·05 ml) were added at 50° with stirring, and the resulting mixture was stirred for 1 hr at 50°.

Ether was then added and the solution was washed with water and aq. $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated under reduced press to an oil which was chromatographed on silica gel. Elution with C_6H_6 gave some unreacted starting material, followed by oily product (V) 280 mg, λ_{max} (MeOH) 294 nm, ε 3·250: ν_{max} 3350, 1500, 1440: 910, 780 and 735 cm^{-1}: δ (CCl_4) 6·46 (3H, m, ArH), 5·25 (1H, t, J = 6 Hz, $C_6H_3(OH)_2CH_2\underline{CH}$=C), 5·07 (6H, m, —CH=C), 3·21 (2H, d, J = 6 Hz, $C_6H_3(OH)_2\underline{CH}_2$—CH=C), 1·98 (24H, bs, CH_2), 1·72 (3H, s, *trans* Me of the first isoprene unit), 1·66 (3H, s, *cis* Me of the terminal isoprene residue), 1·57 (18H, s, *trans* CH_3—C=C): M^+ 586, the mass fragmentation pattern is almost identical, where relevant, with that reported above for the natural mixture of polyprenyl-1,4-benzoquinols.

Acetylation with Ac_2O–pyridine, worked up as usual afforded a *diacetate*, M^+ 670, λ_{max} (MeOH) 266 nm, ε 1100; ν_{max} 1770, δ (CCl_4) CH_3CO— 2·18 and 2·16, which, in GLC (for experimental conditions see Table 1), gave a single peak with retention time (17·01 min) identical to that of component IX derived from the natural mixtures. The synthetic material, when co-chromatographed (SE-30, 1%) with the natural mixtures of the 2-polyprenyl-1,4-benzoquinol acetates could not be separated from the peak corresponding to component IX.

Ozonolysis of furospinosulin-2 (XII) *and -3* (XIII). Each compound (XII and XIII) (50 mg) in EtOAc (10 ml) was ozonized (2% O_3) for 3 hr at −15°. Work up as above gave acetone, identified as the 2,4-dinitrophenylhydrazone by direct comparison (m.m.p., MS and NMR) with an authentic specimen, and succinic and levulinic acids identified as methyl esters by comparison with authentic specimens in GLC (5% SE-30 and 10% DEGS at 100° and 175°, respectively).

Ozonolysis of difurospinosulin (XIV). XIV (10 mg) in EtOAc (5 ml) was ozonized and the ozonide decomposed as above. The mixture was extracted continuously for 5 hr with ether, then concentrated and treated with CH_2N_2. After removal of solvents, the degradation products were analyzed by GLC (5% SE-30 and 10% DEGS at 100° and 175°, respectively) and found to comprise methyl succinate, methyl levulinate and methyl 5-oxo-hexanoate, by comparison with authentic samples.

Ag_2O *Oxidation of prenylated quinol mixtures and of alcohol* VII. Mixtures of polyprenyl-1,4-benzoquinols

(100 mg) (isolated from both sponge samples) in $CHCl_3$–ether (1 : 1) (10 ml) were treated with Ag_2O (250 mg) and $KHSO_4$ (250 mg) with stirring at room temp for 5 min. Filtrn, evapn of the solvent and PLC on silica gel in C_6H_6 gave mixtures of quinones spectroscopically (MS, NMR, IR and UV) identical with the natural quinone mixtures derived from both sponge samples. The hydroxylated -2-octaprenyl-1,4-benzoquinol (VII) (100 mg) when treated with Ag_2O as above yielded the corresponding quinone, M^+ 668, λ_{max} 245, 315 and 440 nm (ε 20,000, 2000, 54): ν_{max} 3350 (b), 1660 and 1600 cm^{-1}: δ (CCl_4) $C\underline{H}_2OH$ 4·02. (Found: C, 81·55: H, 9·90. $C_{46}H_{68}O_3$ requires C, 82·63: H, 10·18%).

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