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ISOAGATHOLACTONE, A DITERPENE OF A NEW STRUCTURAL TYPE FROM THE SPONGE *SPONGIA OFFICINALIS*

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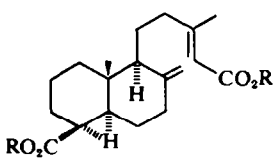
Abstract—A new diterpene, isoagatholactone, isolated from the sponge *Spongia officinalis*, is the first natural compound with the carbon skeleton of isoagathic acid (**2**), the acid-catalyzed cyclization product of agathic acid (**1**). Structure **4** was assigned to isoagatholactone on spectral grounds and chemical correlation with grindelic acid (**5**).

On treatment of agathic acid (**1**; R=H) with formic acid[1] it cyclized to isoagathic acid (**2**; R=H)[1,2]. Surprisingly[3] the carbon skeleton of **2** has not hitherto been encountered in nature although recently a closely related structure has been found in tondiol **3**[4,5] occurring in the marine alga *Taonia atomaria*.

We now report the isolation of a diterpene lactone with the carbon skeleton of isoagathic acid (**2**), and accordingly named isoagatholactone. The new diterpene was isolated from a marine sponge, *Spongia officinalis*,* from which we have also obtained a series of linear C-21 and C-25 furanoterpenes.[6-8] Isoagatholactone **4**, $C_{20}H_{30}O_2$, m.p. 153–155°, $[\alpha]_D$ + 6·3°, shows UV [λ_{max} 222 nm (ϵ = 6,000)] and IR (ν_{max} 1760 and 1690 cm^{-1}) absorption consistent with the presence of an α,β-unsaturated γ-lactone ring. The NMR spectrum includes signals for four t-Me groups (δ0·79, 0·84; 0·88 and 0·94; each 3H, s), an olefinic hydrogen (δ6·69, q, J = 3Hz; H-12) coupled with allylic methylene (δ2·23, bm, H-11, H-11) and an allylic methine (δ2·75, bm, H-14) proton (confirmed by decoupling experiments) and a —CH_2—O— grouping (two triplets, both with J = 9Hz, at δ3·93 and 4·24, each 1H; H-15, H-15), whose protons are coupled with the allylic methine hydrogen at δ2·73, as confirmed by decoupling experiments. It is worth pointing out that coincidentally the value of the coupling constant between the two geminal protons (H-15, H-15) is the same as that of the coupling constants between each geminal proton and the allylic methine hydrogen (H-14). The MS of isoagatholactone was particularly informative, showing a fragmentation pattern (Fig 1) reminiscent of that of methyl isoagathate (**2**, R=Me);[2] the base peak at *m/e* 192, originating by a retro-Diels-Alder process, supports the position of the double bond in the cyclohexane ring C.

The foregoing spectral data together with the molecular formula strongly favoured structure **4** (without sterical implications) for the new diterpene. This was confirmed by chemical interrelation with grindelic acid (**5**, R=H)[9], which also established the stereochemistry of isoagatholactone as shown in **4**. Fig 2 outlines the chemical reactions which allowed us to obtain the alcohol **12** from methyl grindelate (**5**, R=Me). LAH reduction of the latter gave the corresponding alcohol **6**, which on treatment with lithium and ethylamine afforded the dihydroxylabal-8-ene **7**, as described by

*The samples of the sponge containing isoagatholactone proved to be deprived of the furanoterpenes, and the specimens giving furanoterpenes did not contain any of the diterpene lactone. Nevertheless, both samples were identified as *Spongia officinalis* and a comparative analysis revealed only slight morphological differences. Probably the two samples represent different subspecies. The identification of sponges is very difficult, particularly those belonging to the family Spongidae.



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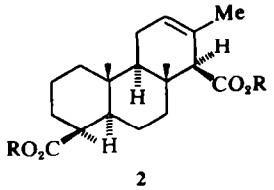

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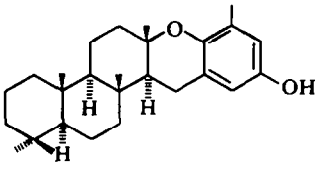

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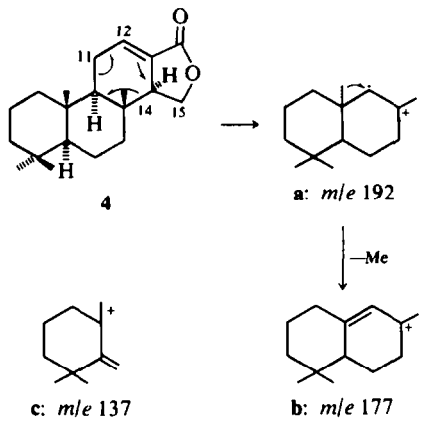


Fig 1. Principal fragments in MS of isoagathalactone.

Panizzi *et al.*[9] Oxidation of **7** with chromium trioxide-pyridine followed by methylation with diazomethane led to the ester **8** which, by dehydration with $POCl_3$-pyridine, gave the two isomeric α,β-unsaturated esters **9** (54%) and **10** (20%). The most significant criteria confirming these assignments are the NMR spectra, especially the chemical shifts of the vinyl Me at C-13, which, as expected[2, 10-12] in **9** resonates at δ 2·15 and in **10** at δ1·91. The α,β-unsaturated ester **9** was converted, in 40% yield, into the tricyclic ester **11** by HCO_2H treatment following the procedure employed by Bory *et al.*[2] to obtain methyl isoagathate (**2**, R=Me) from methyl agathate (**1**, R=Me). The fragmentation pattern in the MS of **11** closely resembles that of both methyl isoagathate (**2**, R=Me)[2] and isoagatholactone **4**, including strong peaks at *m/e* 192 (100%, *a*), 177 (53%, *b*) and 137 (28%, *c*), and the NMR spectrum is very similar, where relevant, to that of methyl isoagathate, showing signals at δ5·59 (H-12; δ5·40 in **2**) and δ2·80 (H-14; δ2·78 in **2**). The *trans*-B/C ring junction and the *quasi*-equatorial carbomethoxyl into **11** were assigned on the assumption that cyclization of **9** follows the same stereochemical path as the cyclization of **1** R=Me)[2]; furthermore, the stereochemical course of this type of cyclization is fairly predictable;[13] it will be recalled that Stork and Burgstabler[14] obtained all-*trans* fused products by acid-catalyzed cyclization of *trans*-monocyclofarnesic acid and similar results were also obtained by Eschenmoser *et al.*[15] using quite different substrates. The β-configuration of the carbomethoxyl group is supported by comparison of the NMR spectrum of **11** with that of the epimer **14** (obtained from the *cis*-α,β-unsaturated ester **10**, by the same acid-catalyzed cyclization) which also defined the stereochemistry of the ester **14**. The *trans*-ring junction in **14** was assigned by analogy as before.[14] The methine proton at C-14 resonates at δ2·80 and 2·37 in **11** and **14**, respectively in accordance with their stereochemistry with respect to the double bond[16] taking into account the diamagnetic anisotropy of the C=C double bond.

LAH treatment of the ester **11** afforded the corresponding alcohol **12**, identical in all respects (TLC, MS, NMR, [α], m.p., m.m.p.) to the alcohol obtained from isoagatholactone (**4**) by LAH reduction, which gave the dihydric alcohol **13** followed by hydrogenolysis of Pd/C (Fig 2).

The *quasi*-axial ester **14**, treated with LAH, also afforded the corresponding alcohol **15**, with physical characteristics completely different from those of its epimer **12**.

EXPERIMENTAL

M.ps are uncorrected. UV and IR spectra were recorded on Bausch and Lomb Spectronic 505 and Perkin-Elmer 257 Infracord Spectrophotometers. NMR spectra were taken on a Varian HA-100 spectrometer (100MHz), TMS as internal standard with $\delta = 0$. Mass spectra were taken on an AEI MS-9. Rotations were measured in $CHCl_3$ solutions. Column chromatography was carried out on silica gel 0·05–0·2 mm (Merck). Analyses were performed in the microanalytical service of our laboratory by Mr. V. Calandrelli. Sponges, collected in the Bay of Naples, were obtained by the supply department of the Zoological Station (Naples).

Isolation of isoagatholactone (**4**). Fresh material (170 g dry weight after extraction) was extracted with cold acetone (×3) for 3 days; after concentration the aqueous residue was extracted with ether (3 × 1 l). The combined ethereal extracts were taken to dryness and the oily residue (7·5 g) was chromatographed on a column of silica gel (300 g) to give, on elution with benzene-ether, 9:1, isoagatholactone (**4**), which was crystallized from MeOH (200 mg), m.p. 153–155°, $[\alpha]_D$ + 6·3° (*c*,3) (Found: C, 79·2; H, 9·8. $C_{20}H_{30}O_2$ requires: C, 79·4; H, 9·9%), λ_{max} 222 nm, ϵ = 6,000 in cyclohexane, ν_{max} ($CHCl_3$) 1760 and 1690 cm^{-1}, δ(CCl_4) 6·69 (1H, q, J = 3Hz; H-12), 4·24 (1H, t, J = 9Hz; H-15), 3·93 (1H, t, J = 9Hz; H-15), 2·75 (1H, bm, H-14), 2·23 (2H, bm, H-11, H-11), 0·79, 0·84, 0·88, 0·94 (each 3H, s, t-Me's), *m/e* 302 (M^+, 5%), 287 (7·5%), 192 (*a*, 100%), 191 (40%), 177 (*b*, 53%), 137 (*c*, 28%).

Hydroxyester (**8**). To a pyridine soln of **7** 4 g in 12 ml, (**7** was prepared from methyl grindelate according to Panizzi *et al.*[10]) excess of Cornforth reagent[7] (chromium trioxide-H_2O-pyridine, 7 g-4 ml-70 ml) was added. The mixture was allowed to react at room temp overnight and then diluted with 0·1N HCl and the aqueous phase was extracted with ether. The combined ethereal extracts were dried over $NaSO_4$ and evaporated *in vacuo* to an oil, which was treated with excess ethereal diazomethane. After removal of solvent the residue was chromatographed on silica gel (300 g) to give, by elution with benzene-ether, 95:5, 2·2 g of **8**, oil, $[\alpha]_D$ + 37·2° (*c*, 2·6) (Found: C, 74·7; H, 10·2. $C_{21}H_{36}O_3$ requires: C, 75·0; H, 10·7%), ν_{max} (liquid film) 3400 and 1720 cm^{-1}, δ($CDCl_3$) 3·70 (3H, s; OCH_3), 2·50 (2H, ABq, J = 15Hz, H-14, H-14), 1·55 (3H, s; CH_3—C=C), 1·26 (3H, s; CH_3—C—O), 0·93, 0·87 and 0·81 ppm (each 3H, s; t-Me's), M^+/*e* 336.

α,β-Unsaturated esters **9** *and* **10**. A soln of **8** (2 g) in dry pyridine (40 ml) was stirred and cooled in an ice bath and treated with 7 ml $POCl_3$. After 10 min the cooling bath

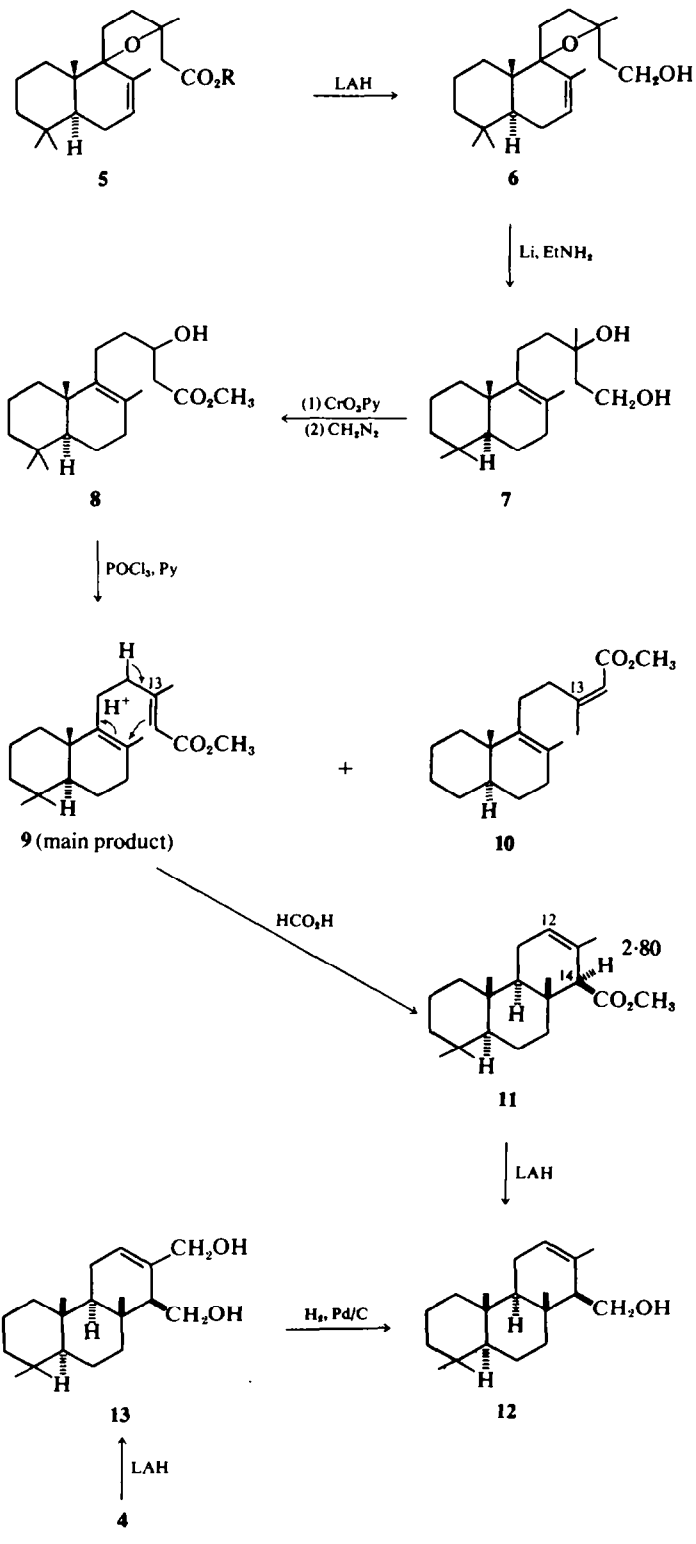
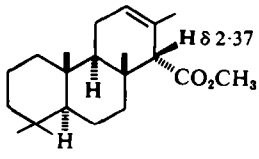


Fig 2. Chemical correlation of isoagatholactone with grindelic acid.



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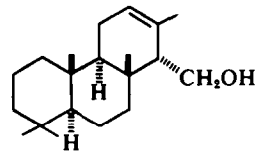

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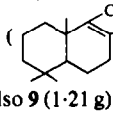
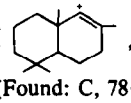
was removed and the mixture was stirred at room temp for an additional 2 hr. The resulting mixture was poured into ice-water and extracted with ether. The combined ether extracts were washed with 2N HCl, water, dried over Na_2SO_4 and evaporated to an oil which was carefully chromatographed on SiO_2 (150 g) column in light petroleum-benzene, 6:4. Thus we obtained; **10** (0·36 g), oil, $[\alpha]_D$ + 55·5° (*c*, 2·5) (Found: C, 78·8; H, 10·3. $C_{21}H_{34}O_2$ requires: C, 79·2; H, 10·7%), λ_{max} 219 nm, ϵ = 9,400 in MeOH, ν_{max} (liquid film) 1710 and 1635 cm^{-1}, $\delta(CCl_4)$ 5·54 (1H, s; CH=C), 3·61 (3H, s; OMe), 1·91 (3H, s; Me—C=C—CO_2Me), 1·63 (3H, s, Me—C=C), 0·95, 0·89 and 0·83 ppm (each 3H, s; t-Me's), *m/e* 318 (M^+, 33%), 303 (M^+—Me, 12%), 205 ( , 100%) and 191 ( , 33%). Also **9** (1·21 g), oil, $[\alpha]_D$ + 50·8° (*c*, 3) (Found: C, 78·9; H, 10·2. $C_{21}H_{34}O_2$ requires: C, 79·2; H, 10·7%), λ_{max} 221 nm, ϵ = 14,300 in MeOH, ν_{max} (liquid film) 1715 and 1640 cm^{-1}, $\delta(CCl_4)$ 5·59 (1H, s; CH=C), 3·60 (3H, s; O), 2·15 (3H, s; Me—C=C—CO_2Me), 1·56 (3H, s; Me—C=C), 0·94, 0·88 and 0·82 ppm (each 3H, s; t-Me's), *m/e* 318 (M^+, 17%), 303 (M^+–Me, 8%), 205 (100%) and 191 (22%).

Ester **11**. A soln of 1·1 g of **9** in 98% aqueous formic acid (20 ml) was allowed to stand at 70° for 1 hr according to Bory *et al*[3]. After removal of formic acid *in vacuo*, the residue was heated under reflux in MeOH (20 ml) and water (20 ml) containing KOH (2·3 g) for 3 hr. After evapn of the MeOH, the soln was extracted with ether. The combined ethereal extracts were dried over Na_2SO_4 and evaporated to a solid which was crystallized from MeOH (**11**, 360 mg), m.p. 103–105°, $[\alpha]_D$–50·4° (*c*, 2·6) (Found: C, 79·1, H, 10·5. $C_{21}H_{34}O_2$ requires: C, 79·2; H, 10·7%), ν_{max} ($CHCl_3$) 1720 cm^{-1}, $\delta(CDCl_3)$ 5·52 (1H, *m*; CH=C), 3·66 (3H, s; OMe), 2·80 (1H, bs; H-14), 1·60 (3H, s; Me—C=C), 0·93, 0·89, 0·85 and 0·81 ppm (each 3H, s; t-Me's), *m/e* 318 (M^+, 35%), 303 (M^+–Me, 10%), 192 (*a*, 100%), 191 (44%), 177 (*b*, 55%) and 137 (10%).

Ester **14**. The *cis*-α,β-unsaturated ester **10** (0·5 g) when treated with formic acid and the rn mixture worked up as before, afforded 150 mg of **14**, m.p. 88–90° from MeOH, $[\alpha]_D$ + 169° (*c*, 1·2) (Found: C, 78·9; H, 10·4. $C_{21}H_{34}O_2$ requires: C, 79·2; H, 10·7%), ν_{max} ($CHCl_3$) 1715 cm^{-1}, $\delta(CCl_4)$ 5·50 (1H, m; CH=C), 3·61 (3H, s; OMe), 2·37 (1H, bs; H-14), 1·57 (3H, s, Me—C=C), 0·90, 0·88 and 0·83 ppm (each s, integrating together for 12 H; t-Me's), *m/e* 318 (M^+, 100%), 303 (M^+-Me, 35%), 205 (35%), 192 (*a*, 55%), 191 (45%), 177 (*b*, 45%) and 137 (25%).

Alcohol **12**

(a) *From ester* **11**. To a soln of **11** (300 mg) in 8 ml dry ether, a suspension of 180 mg of LAH in 3 ml dry ether was added dropwise with stirring. The mixture was stirred under reflux for 3 hr. Work-up afforded 270 mg of crystalline compound which was recrystallized from MeOH to give **12** (200 mg) as colourless plates, m.p. 125–126°, $[\alpha]_D$–9° (*c*, 1) (Found: C, 82·42; H, 11·50% $C_{20}H_{34}O$ requires: C, 82·69; H, 11·80%), $\delta(CDCl_3)$ 5·51 (1H, bt, J = 4–5Hz; CH=C), 3·81 (2H, m; —CH_2O), 1·79 (3H, bs, Me—C=C), 0·90, 0·85 and 0·82 (each singlet, integrating together for 12 H; t-Me's), *m/e* 290 (M^+, 29%), 192 (*a*, 100%), 191 (40%), 177 (*b*, 60%), 137 (*c*, 10%).

(b) *From isoagatholactone* (**4**). Isoagatholactone (**4**, 100 mg) was refluxed with 70 mg LAH in dry ether (3 ml) for 1 hr and treated as usual, giving 90 mg of a solid which was purified by SiO_2 column chromatography (10 g; eluent: benzene-ether, 6:4) and then by crystallization from 80–100° petrol to give 70 mg of **13** as colourless needles, p.f. 159–161°, $[\alpha]_D$–16·5° (*c*, 3) (Found: C, 78·1; H, 10·9. $C_{20}H_{34}O_2$ requires: C, 78·4; H, 11·1%), $\delta(CDCl_3)$ 5·77 (1H, m; CH=C), 4·16 (2H, ABq, δA-δB = 30Hz, J = 12Hz; =C—CH_2OH), 3·86 (2H, bm; HC—CH_2OH), 0·87 (6H, s; t-Me's), and 0·79 and 0·72 (each 3H, s; t-Me's), *m/e* 306 (M^+, 17%), 288 (14%), 275 (6%), 258 (71%), 243 (11%), 192 (*a*, 100%), 177 (*b*, 91%). **13** (60 mg) in 6 ml AcOH was hydrogenated over 20 mg of 5% Pd/C at room temp and atmospheric pressure for 1 hr. Filtn, evapn and SiO_2 (5 g) column chromatography (benzene-ether, 9:1) gave 20 mg of crystalline **12** which, after crystallization from MeOH, melted at 124–125°, $[\alpha]_D$–10° (*c*, 1·4) and NMR and MS were totally identical with those of alcohol **12** derived from the ester **11**.

Alcohol **15**. Ester **14** (150 mg) was treated with LAH as above. Work-up as usual gave 92 mg of **15**, crystallized from 80–100° petrol, m.p. 107–109°, $[\alpha]_D$ + 52·6° (*c*, 1·2) Found: C, 82·21; H, 11·5. $C_{20}H_{34}O$ requires: C, 82·69; H, 11·8%), $\delta(CDCl_3)$ 5·53 (1H, bt, J = 4–5Hz; CH=C), 3·81 (2H, d, J = 4Hz; —CH_2O), 1·72 (bs, CH_3—C=C), 0·9 (s, integrating for 9 H; t-Me's) and 0·85 (3H, s, t-Me's); *m/e* 290 (M^+, 27%), 275 (14%), 272 (8%), 259 (10%), 257 (8%), 192 (*a*, 100%), 191 (65%), 177 (*b*, 100%) and 137 (*c*, 15%).

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