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Stypolactone, an interesting diterpenoid from the brown alga *Stypopodium zonale*

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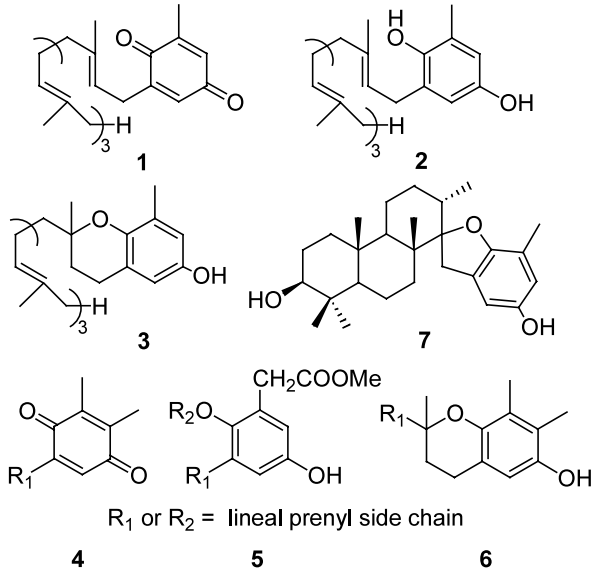
Abstract—A new diterpenoid of mixed biogenesis, stypolactone **8**, containing a tricyclic terpenoid moiety which in combination with its spirolactone ring, leads to a novel carbon skeleton, has been isolated from the brown alga *Stypopodium zonale*. It's structure and relative stereochemistry were established by spectroscopic evidence and biogenetic considerations.

Among the brown algae of Dictyotaceae family the genera *Cystoseira*, *Sargassum*, *Bifurcaria*, *Taonia*, *Desmarestia*, *Halidrys*, *Cystophora*, *Lobophora* and *Stypopodium* possess the ability to produce diterpenes of mixed biogenesis that exhibit interesting biological activity such as: ichthyotoxic,[1] insecticidal,[2] antitumoral,[3,4] antiviral,[4] endothelin antagonists,[5] tyrosine kinase inhibitor,[6] antimicrobial[7] and antimitotic.[8] For a given species, depending on location and season, qualitative and quantitative chemical variation has been observed.[9–11] These genera have a common feature: the non-isoprenic moiety of all such diterpenoids is a C_7 or the less common C_8 benzoquinone or hydroquinone unit.

Only species of genus *Sargassum* are able to produce metabolites with both C_7[3,12,13] and C_8[6,12] aromatic rings (structural types from **1** to **5**),[1,3,6,14] whereas a unique metabolite **6**[15] containing a C_8 unit was isolated from *Lobophora papenfussii*, and no diterpenoid based on the more common C_7 toluhydroquinone-ring moiety has been isolated from this genus.

However, variation in the isoprenic portion of the skeletal structures appears to be genus-dependent. For example, benzo- and hydroquinone-derived metabolites with a linear prenyl side chain are common in all[16] except *Taonia* and *Bifurcaria*.

In this work we report on an unique diterpene stypolactone **8** isolated from the brown alga *Stypopodium zonale* (L.) Lamouroux (Dyctiotaceae). The compound possesses a structurally tricyclic diterpene moiety in combination with an unprecedented non-isoprenic C_2-unit forming part of a spirolactone ring.



Compound **8** was isolated as an amorphous white powder.[17] The EIMS spectrum showed a peak at m/z 362 that corresponds to the molecular formula $C_{22}H_{34}O_4$ $[M]^+$ (HRMS) (six unsaturation degrees). Absorbances for hydroxyl and carbonyl groups were observed at 3362 and 1723 cm^{-1}, respectively, in the IR

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spectrum. The ^{13}C NMR spectrum (Table 1) displayed signals for 22 carbons; multiplicities of the carbon signals were determined from the DEPT spectra. Five methyls; six methylenes; five methines (one olefinic and one bearing oxygen) and six nonprotonated carbons (one bearing oxygen, one carbonyl, and one olefinic). The ^1H NMR spectrum showed a signal for one olefinic proton at δ 6.10 (s) and a proton geminal to oxygen at δ 3.13 (dd, $J=5.1$, 11.2 Hz). In the upfield region appeared signals for five methyl groups at δ 1.10 (3H, s), δ 0.93 (3H, s), δ 0.88 (3H, s), δ 0.75 (3H, s) and δ 0.63 (3H, d, $J=6.6$ Hz).

The presence of a secondary methyl group [δ_C 16.1; δ_H 0.63 (3H, d, $J=6.6$ Hz)] and a quaternary carbon at δ 93.8 suggested a structure with a terpenoid portion similar to that of stypodiol **7**.[1] However, compound **8** has only 22 carbons, thus the non-isoprenic residue has five carbon atoms less than the corresponding toluhydroquinone moiety of stypodiol. No signals for the benzylic methylene or aromatic methyl group characteristic of this type of skeleton were observed.

Stypolactone **8** was insoluble in $CDCl_3$. For the purpose of comparing the ^{13}C NMR chemical shifts of **8** with those of stypodiol **7**, the ^1H and ^{13}C NMR of **7** were therefore recorded in methanol-d_4.

From Table 1 it can be observed that the diterpene portions of compounds **7** and **8** have similar ^1H and ^{13}C chemical shifts with the exception of the atoms close to the non-terpenoid moiety, suggesting that they possess the three fused rings with identical features. This was also corroborated by the study of the 2D NMR spectral data of **8**. HSQC and HMBC data allowed us to establish the linkage, as depicted in **8**, between the three discrete **a**–**c** fragments detected by a ^1H–^1H COSY NMR spectrum (Fig. 1).

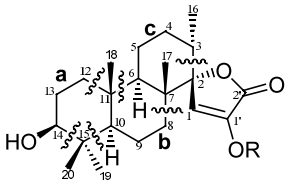


Figure 1.

Table 1. ^1H and ^{13}C NMR data of compounds **7**, **8** and **8a** [500 MHz, δ ppm, (J) Hz, CD_3OD]

	7		**8**			**8a**	
#	δ_H	δ_C	δ_H	δ_C	HMBC	[a]δ_H	[a]δ_C
1	2.72 d (16.5); 3.18 d (16.4)	36.5	6.10 s	122.5	C=O, C-2	7.07 s	136.5
2		96.0		93.8			93.1
3	1.68 m	38.7	2.13 m	34.9	C-16	2.15 m	33.0
4	1.41 m	34.3	1.72 m	33.0		1.45 m	31.0
	1.35 dd (4.2, 13.1)		1.35 dd (4.5, 12.7)				
5	1.67 m	28.5	1.69 m	28.3	C-6	1.65 m	27.1
	1.51 m		1.56 m			1.56 m	
6	1.54 m	53.2	1.42 dd (2.2, 12.4)	53.4	C-7, C-11	1.50 m	50.9
7		44.0		43.6			42.8
8	1.50 m	32.7	1.13 d (4.5)	35.4	C-17	1.24 m	33.7
			1.25 dt (3.8, 13.0)				
9	1.59 m; 1.49 m	21.8	1.63 m; 1.48 m	21.8		1.62 m; 1.44 m	20.2
10	0.73 m	56.9	0.73 d (2.3)	56.5	C-18	0.81 m	54.1
11		38.6		38.9			37.4
12	1.74 m	40.1	1.74 m	40.0		1.70 m	38.0
	1.00 m		1.00 dd (4.5, 12.8)		C-18	1.01 m	
13	1.46 m	19.3	1.59 m; 1.53 m	19.3		1.52 m; 1.36 m	17.6
14	3.13 dd (5.1, 11.3)	80.0	3.13 dd (5.1, 11.2)	79.8	C-19, C-20	3.19 dd (4.7, 11.6)	78.5
15		40.3		40.3			38.7
16	0.63 d (6.5)	16.4	0.63 d (6.6)	16.1	C-2, C-3, C-4	0.69 d (6.6)	16.2
17	0.90 s	18.0	1.10 s	19.6	C-2, C-6, C-7, C-8	1.12 s	18.8
18	0.86 s	17.2	0.88 s	17.2	C-6, C-10, C-12	0.85 s	17.6
19	0.95 s	29.0	0.93 s	28.9	C-10, C-14, C-15, C-20	0.94 s	27.8
20	0.73 s	16.4	0.75 s	16.4	C-10, C-14, C-15, C-19	0.75 s	15.3
1′		128.4		145.1			137.3
2′		154.7		172.3			167.3
3′		119.4					
4′	6.30 s	116.4					
5′		151.5					
6′	6.33 s	109.8					
7′	2.08 s	15.9					
O*H*			3.33 s				

[a] $CDCl_3$.

The molecule has six degrees of unsaturation and the presence of one olefin and one carbonyl implies that the molecule must have four rings. Chemical shifts of the remaining carbons C-1 (δ 122.5); C-2 (δ 93.8); C-1′ (δ 145.1) and C-2′ (δ 172.3) indicate that the fourth ring must be an α,β-unsaturated lactone. Chemical shift arguments allowed us to place the olefinic proton at C-1 and a hydroxyl group at C-1′. The presence of the hydroxyvinyl group was confirmed by partial acetylation of **8** to obtain **8a**. This completes the planar structure of **8**.

2D NOESY experiments on **8** show a clear NOE effect between Me-20 and Me-18 and also between Me-19 and H-14 and H-10. These data suggested that C-10/C-11 ring fusion is *trans* and placed the hydroxyl group at C-14 and Me-18 and Me-20 on the same β face of the molecule. A NOE effect between Me-17 and Me-18 and H-3 placed Me-16 and Me-17 on opposite faces of the molecule. Also NOE between H-6 and H-10 indicates the C-6/C-7 ring fusion must be *trans*. Finally, the NOE effect between H-1 and Me-17 established the relative stereochemistry around the spiro carbon atom, with C-1 and Me-17 on the β face of the molecule, confirming also the position of the olefinic proton (Fig. 2).

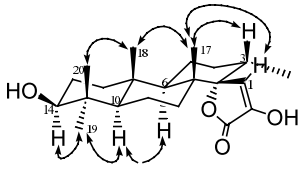


Figure 2. Selected NOE for stypolactone **8**.

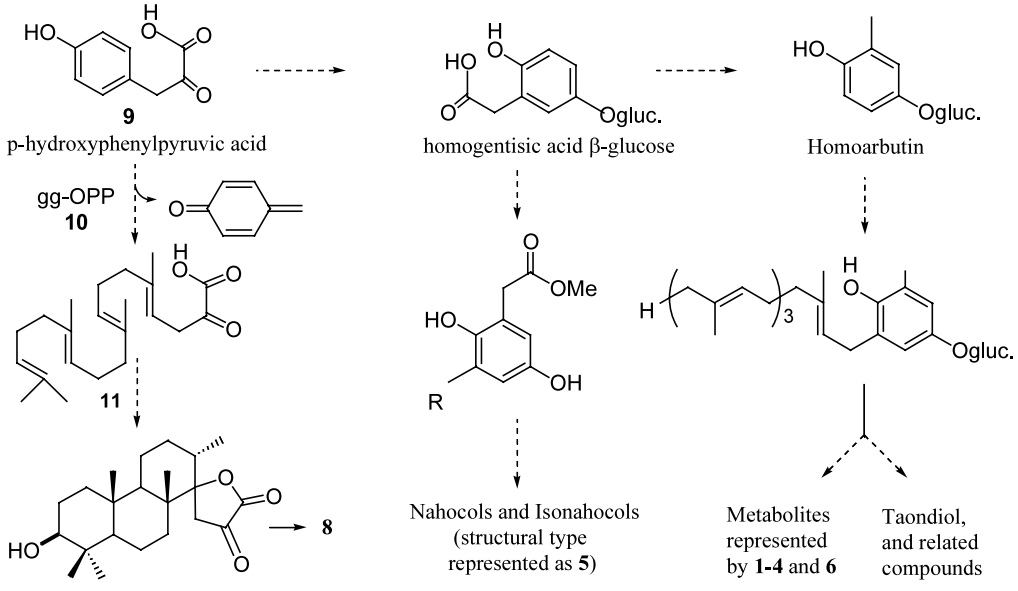
It is well known that stypotriol is quite rapidly air-oxidized to stypoldione,[18] hence it is not unreasonable to suggest that the α-keto lactone of stypolactone is derived biosynthetically or by air-oxidation from an aromatic ring. However, from a careful revision of the literature along the 31 years elapsed from the isolation of the first related naturally occurring metabolite taondiol,[19] we could not find any metabolite with a structural feature indicative of such an air-oxidative aromatic ring degradation.

Biosynthetically, the pathway proposed for the biosynthesis of plastoquinones and tocopherols in higher plants appears to be plausible for the biosynthesis of **8**.[20] Thus, the non-terpenoid moiety of stypolactone **8** could be derived from nucleophilic attack of the C_2-α-ketoacid residue of *p*-hydroxyphenyl piruvic acid to the geranylgeraniol pyrophosphate (gg-OPP), **10**, to give **11**. The cyclization induced by ring opening of the terminal epoxide derivative of **11** could account for the formation of stypolactone **8**.

In this way, all naturally occurring metabolites of mixed biogenesis from the Dictyotaceae family could be explained from a common precursor **9** following the sequence: *p*-hydroxyphenyl pyruvic acid→ homogentisic acid β-glucose→homoarbutin, as it is depicted in Scheme 1.

Although compound **8** structurally resembles to stypodiol **7** it differs greatly from stypodiol from the biogenetic point of view.

Compound **8** exhibits a weak in vitro cytotoxicity against A-549 (human lung carcinoma) and HT-29 and H-116 (human colon carcinoma) cell lines, displaying IC_{50} values of >25.0 μg/ml, in each case.



Scheme 1.

Acknowledgements

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17. $[\alpha]_D^{25} = -67$ (*c* 0.06, CH_3OH); IR (film) ν_{max} 3362, 2855, 1723, 1657, 1386 cm^{-1}; EIMS *m/z* 362 $[M]^+$ (7); HREIMS $[M]^+$ 362.245 (calcd for $C_{22}H_{34}O_4$, 362.245); ^1H and ^{13}C NMR, see Table 1.
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