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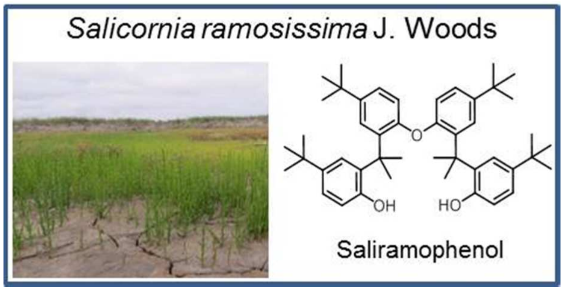

A unique skeleton-type compound with four *t*-butyl groups, named saliramophenol, was identified in *Salicornia ramosissima* J. Woods aerial parts.

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Saliramophenol, an unprecedented natural *t*-butylphenol derivative from *Salicornia ramosissima* J. Woods†

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V. M. S. Isca,[a] A. M. L. Seca,[a,b] D. C. G. A. Pinto,[a*] H. Silva[c] and A. M. S. Silva[a]

Saliramophenol (1), a unique skeleton-type compound with four *t*-butyl groups, was isolated from aerial parts of *S. ramosissima* J. Woods. The structures of the isolated compound was elucidated by detailed NMR spectroscopic methods and mass spectrometry data.

The interest in *Salicornia* species (Chenopodiaceae family) is growing due its versatile commercial products with great nutritional value,[1,2,3] along with its ethnopharmacological applications.[3] Simultaneously *Salicornia* species are a source of bioactive metabolites.[3] *Salicornia ramosissima* J. Woods is an annual halophyte usually erect, 3-40 cm tall, fleshy, with stem articulated and branched apparently leafless,[4] included in the species aggregate *S. europaea* agg. and that grows naturally on the salt marsh of Europe.[5] From the chemical profile point just recently its lipophilic profile was characterized[6] and some of its phenolic constituents were identified.[7] As a part of our continuing search for structurally interesting natural products from plants, the dichloromethane extract from aerial parts of *S. ramosissima* was phytochemically investigated and as a result a new natural *t*-butylphenol derivative, named saliramophenol (**1**) (Fig. 1) was isolated from this halophyte collected in Marina dos Puxadoiros (40º 39’ 23’’ N, 8º 40’ 35’’ W), Ria de Aveiro, Portugal (yield 0.00055 % of dried plant). Herein, the isolation and structure elucidation of **1** is described.

The compound (**1**) 2,2'-[oxybis(3-(*tert*-butyl)-6,1-phenylene]bis(propane-2,2-diyl)bis(4-*tert*-butyl)phenol], named saliramophenol, is a new natural phenol derivative with four *tert*-butyl groups. It was isolated as a pale yellow oil and have the molecular formula of $C_{46}H_{62}O_3$, which was established on the basis of its HRMS-ESI(+) peak at *m/z* 663.4773 $[M+H]^+$ (calcd for $C_{46}H_{63}O_3$, 663.4772). Additionally, MS/MS analysis showed consecutive losses of *m/z* = 56 from the signal with *m/z* = 663 ($[M+H]^+$), which are consistent with losses of the four *t*-butyl groups. Another structu evidence is shown by further MS/MS analysis of the signal with *m/z* = 685 ($[M+Na]^+$). In this case not only is evidenced the loss of the butyl groups but also a signal with m/z = 251 which corresponds to the mass of a fragment with two aromatic rings and thus indicating the ether linkage between carbons C-1’ and C-1’’ occurred.

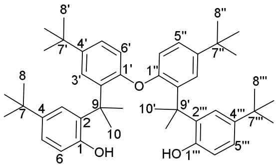

Fig. 1 Chemical structure of saliramophenol (**1**).

The ^{13}C NMR data (Table 1) and the DEPT spectrum showed only four non-equivalents sp^3 carbons (two methylic and two quaternary carbons) and eight non-equivalents sp^2 carbons (three CH and fi deprotonated aromatic carbons). These features reveal a high degree of symmetry in the compound (**1**) chemical structure. The compound also possesses, two quasi-equivalents 1,2,4-trisubstituted aromatic rings, as evidenced by the presence of the downfield ^1H NMR signals at δ_H 7.13, as a double doublet (*J*= 2.5 and 8.6 Hz), at δ_H 7.53 as a doublet (*J*= 8.6 Hz) and at δ_H 7.35 and 7.36, as two doublets (*J*= 2.5 Hz), the coupling each other was confirmed by the COSY NMR spectrum.

The presence of four equivalent *t*-butyl groups attached to the aromatic rings were deduced from the following spectroscopic evidence: i) a singlet at δ_H 1.28 (integral proportional to 36 protons) showing $^1J_{H-C}$ correlation (HSQC spectrum) with the signal at δ_C 31.4, corresponding to nine methyl groups (table 1); ii) this ^1H NMR signal showed connectivities (HMBC spectrum) with the signals at δ_C 31.4, 34.5 and 147.0 (Fig. 2 a), corresponding, respectively, to the resonance of the methylic carbons (C-8, C-8’, C-8’’ and C-8’’’), aliphatic quaternary carbons (C-7, C-7’, C-7’’ and C-7’’’) and deprotonated aromatic carbons (C-4, C-4’, C-4’’ and C-4’’’).

The ^1H NMR spectrum also showed a singlet at δ_H 1.3, corresponding to the resonances of the protons from 4 equivale methyl groups (δ_C 30.2), and that exhibits HMBC connectivities wit the ^{13}C NMR signals at δ_C 30.2, δ_C 34.8 (aliphatic quaternary carbo

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[a] Department of Chemistry & QOPNA, University of Aveiro, 3810-193 Aveiro, Portugal. E-mail: diana@ua.pt; Fax: +351 234 370084; Tel: +351 234 370714

[b] DCTD, University of Azores, Rua Mãe de Deus, 9501-801 Ponta Delgada, Portugal

[c] Department of Biology, & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal.

†Electronic Supplementary Information (ESI) available: 1D and 2D NMR, MS and HRMS spectra of saliramophenol (**1**) and detailed experimental procedure. See DOI: 10.1039/x0xx00000x

and δ_C 138.4, 138.5 (Fig. 2 b), showing that the two *t*-butylphenol units are linked by a isopropyl-type group.

The most downfield ^{13}C NMR signals (δ_C 147.6 and 147.7) corresponding to two non-equivalent oxygen-bearing carbons in position C-1, C-1''' and C-1', C-1''. The FTIR absorption bands at ν_{max} 3443 cm^{-1} suggests the presence of OH group bearing to aromatic moiety.

Table 1. ^{1}H and ^{13}C NMR chemical shifts for Saliramophenol (**1**) in CDCl$_3$

Position	**Compound 1**	
	δ_H (*J* in Hz)	δ_C, type
1		147.6, C^a
2		138.5, C^b
3	7.35 (1H, d, 2.5)^c	120.4, CH
4		147.0, C
5	7.13 (1H, dd, 2.5, 8.6)	124.0, CH
6	7.53 (1H, d, 8.6)	119.1, CH
7		34.5, C
8	1.28 (9H, s)	31.4, CH$_3$
9		34.8, C
10	1.33 (6H, s)	30.2, CH$_3$
1'		147.7, C^a
2'		138.4, C^b
3'	7.36 (1H, d, 2.5)^c	120.4 CH
4'		147.0, C
5'	7.13 (1H, dd, 2.5, 8.6)	124.0, CH
6'	7.53 (1H, d, 8.6)	119.1, CH
7'		34.5, C
8'	1.28 (9H, s)	31.4, CH$_3$
9'		34.8, C
10'	1.33 (6H, s)	30.2, CH$_3$
1''		147.7, C^a
2''		138.4, C^b
3''	7.36 (1H, d, 2.5)^c	120.4, CH
4''		147.0, C
5''	7.13 (1H, dd, 2.5, 8.6)	124.0, CH
6''	7.53 (1H, d, 8.6)	119.1, CH
7''		34.5, C
8''	1.28 (9H, s)	31.4, CH$_3$
1'''		147.6, C^a
2'''		138.5, C^b
3'''	7.35 (1H, d, 2.5)^c	120.4, CH
4'''		147.0, C
5'''	7.13 (1H, dd, 2.5, 8.6)	124.0, CH
6'''	7.53 (1H, d, 8.6)	119.1, CH
7'''		34.5, C
8'''	1.28 (9H, s)	31.4, CH$_3$

$^{a, b, c}$ Those marked with the same symbol may be exchanged

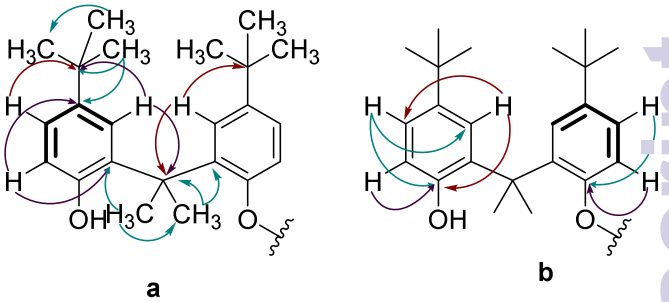

Fig. 2 Coupling deduced by the COSY spectrum (bold line) and HMBC connectivities (→), observed for compound **1**.

All these spectroscopic data and other HMBC connectivities (Fig. 2a and b) are only compatible with the structure presented here to the compound **1**, saliramophenol (Fig. 1).

Natural compounds with *t*-butyl groups are described in the literature[8,9] and *t*-butylphenol is a known commercial product used in the synthesis of resins so can be widespread in the environment.[10,11] Though saliramophenol **1**, the new natural compound with a unique carbon skeleton, here reported cannot be an artifact since the type of bond between the aromatic rings cannot be formed during the isolation and/or purification procedures. So it must be biosynthesized using *t*-butylphenol as substrate and with the intervention of enzymes. It seems that saliramophenol **1** could be one of the first examples of compounds isolated from plants that are a result of biogenetic pathways using anthropogenic substrates.

There are examples of biologically active compounds possessing *t*-butyl phenol units, for example succinobucol is an synthetic antiatherosclerotic drug proved to have antihyperglycemic activity and is in Phase III clinical trials.[12] There are also evidences that the *t*-butyl group seems to play an essential role in the platelet activating factor receptor antagonists activity.[13] However, these unique structure in a natural compound most likely is due to the environmental stress of the *S. ramosissima* habitat (*e.g.* salinity, water supply).

In summary, saliramophenol **1** is the first example of a natural compound with a dimeric structure of two *t*-butylphenol units linked by carbon-carbon skeleton *i*-propyl-type. The isolation and unequivocal structural elucidation of **1** has added a completely new skeleton to the already large and varied family of chemical structures obtained from natural resources. Further investigations regarding compound **1**'s biological effects are in progress.

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