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Tragoponol, a dimeric dihydroisocoumarin from *Tragopogon porrifolius* L.

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ABSTRACT

A phytochemical re-investigation of *Tragopogon porrifolius* L. (Asteraceae) yielded (7*S*,15*S*)-2,4,12-trihydroxy-7-(4-hydroxyphenyl)-10-methoxy-15-(4-methoxyphenyl)-7,8,15,16-tetrahydrodibenzo[*c*,*i*][1,7]dioxacyclododecine-5,13-dione, named tragoponol, a dimeric dihydroisocoumarin.
The compound, which represents the first of its kind, is comprised of the open-chained forms of two different mono-methoxylated dihydroisocoumarin moieties, scorzocreticin and hongkongenin, which are connected via two ester bonds to form a macrolide with two lactone moieties featuring a 12-membered ring. The structure of the nearly symmetrical compound was established by HR mass spectrometry, CD measurements, and extensive 1D and 2D NMR experiments.

Tragopogon porrifolius L. (Asteraceae), commonly known as white salsify, is an annual or a biennial herb of 30–125 cm height with lilac to reddish-purple ligules. The species is indigenous to the Eastern and central Mediterranean region and Asia Minor. The roots and young shoots are used as vegetables.[1]

Compound **1** was initially isolated from rootstocks collected N Villanueva de la Concepcion/Malaga/Andalusia/Spain in April 2003 (coordinates: N 36°56′32″, W 04°32′08″; alt.: 800 m). A voucher specimen is preserved in the Herbarium of the Institut für Pharmazie, Innsbruck (voucher code: CZ-20030418C-1).[2] Besides two simple bibenzyls and the dihydroisocoumarin thunberginol C, this plant material also yielded dihydroisocoumarin glucosides **2** and **3**.[2]

For re-isolation of compound **1**, rootstocks of *T. porrifolius* collected in April 2007 in Sicily near Castelbuono/Palermo/Sicily/Italy (CZ-20070416C-1, N 37°56′05″; E 14°05′46″; alt.: 320 m a.m.s.l.) were used. A voucher specimen was deposited in the Herbarium of the Universität Innsbruck, voucher code: IB #26888.

Tragoponol **1** (1.89 mg) was isolated from the EtOAc layer (3.32 g) of the methanolic extract (36.6 g) of subaerial parts (128 g) Spanish plants of *T. porrifolius* by silica gel 60 (230–400 mesh) column chromatography (CC) using a gradient of CH_2Cl_2 and MeOH and a successive separation step of a fraction (171 mg) enriched in **1** by Sephadex LH-20 CC using MeOH as the eluant. Moreover, tragoponol **1** (1.53 mg) was re-isolated from the EtOAc layer (18.0 g) of the methanolic extract (121 g) of subaerial parts (320 g) of *T. porrifolius* from Sicily by silica gel CC using a gradient of petrol ether, CH_2Cl_2 and MeOH and a successive fractionation step of a fraction (188 mg) enriched in **1** by Sephadex LH-20 CC using MeOH as the eluant. In crude extracts of both accessions compound **1** was detected by HPLC-MS. Thus, we can exclude the possibility that the compound is an artifact from the isolation procedure.

HRMS[3] of compound **1** revealed a molecular formula of $C_{32}H_{28}O_{10}$. ^{1}H NMR and ^{13}C NMR data (Table 1) indicated that **1** consisted of two stilbenoid moieties and two methyl groups. The stilbenoid moieties closely resembled dihydroisocoumarins isolated from *T. porrifolius* in a previous investigation.[2] Extensive 2D NMR spectroscopy using high paramagnetic field forces and various solvents [MeOH-d_4 (Table 1), acetone-d_6 (data not shown), DMSO-d_6 (Table 3)] verified this assumption and established that one methyl group was in *para*-position to the carboxyl group of the first dihydroisocoumarin moiety and the second was attached to the oxygen of the mono-hydroxylated aromatic ring of the other dihydroisocoumarin moiety. Thus, the two monomeric subunits of compound **1** were identified as hongkongenin (7-*O*-methyldihydroisocoumarin)[4] and scorzocreticin (4′-*O*-methyldihydroisocoumarin),[5] or their open-chained analogues, respectively (Fig. 1). Moreover, analysis of the circular dichroism of **1** revealed that both subunits were *S*-configured and thus had the same stereochemistry as established for compound **3** (Fig. 2).[2]

Establishing the exact linkage of the two stilbenoid moieties proved to be more challenging than initially expected. The main problem was that no unambiguous direct HMBCs correlations

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Table 1
NMR data of tragoponol **1**[a]

Position	6-*O*-Methyldihydroisocoumarin moiety		Position	4′-*O*-Methyldihydroisocoumarin moiety	
	^{1}H NMR	^{13}C NMR		^{1}H NMR	^{13}C NMR
1		171.52	1″		171.38
3	5.513 1H, m*	81.91	3″	5.531 1H, m*	81.61
4	3.306 1H, m*	35.60	4″	3.264 1H, m*	35.67
	3.085 1H, m*			3.041 1H, m*	
4a		143.30	4a″		143.41
5	6.430 1H, br s	106.83	5″	6.264 1H, br s	107.88
6		167.56	6″		166.50
7	6.409 1H, d (2.0)	100.37	7″	6.226 1H, d (2.0)	102.15
8		165.57	8″		165.62
8a		102.45	8a″		101.33
1′		130.45	1‴		131.86
2′	7.324 1H, d (8.5)	128.85	2‴	7.421 1H, d (8.5)	128.73
3′	6.822 1H, d (8.5)	116.11	3‴	6.966 1H, d (8.5)	114.78
4′		159.04	4‴		161.36
5′	6.822 1H, d (8.5)	116.11	5‴	6.966 1H, d (8.5)	114.78
6′	7.324 1H, d (8.5)	128.85	6‴	7.421 1H, d (8.5)	128.73
6-*O*CH$_3$	3.845 3H, br s	55.93	4‴-*O*CH$_3$	3.813 3H, br s	55.50

[a] Measured in CD_3OD at 800 and 200 MHz, respectively, and referenced to solvent residual and solvent signals at 3.310 ppm (^{1}H NMR) and 49.00 ppm (^{13}C NMR), respectively.
* Overlapping signals.

Table 2
NMR shift values of related dihydroisocoumarins from the literature[a]

Position	Thunberginol C (Ref. 9)[b]		Hongkongenin (Ref. 4)[c]		Scorzocreticin (Ref. 5)[d]	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1		169.4		169.4		169.9
3	5.54 1H	79.7	5.60 1H	79.9	5.42 1H	80.1
4	3.24 1H	33.6	3.34 1H	33.7	3.19 1H	35.1
	3.03 1H		3.08 1H		2.94 1H	
4a		142.2		142.2		141.4
5	6.30 1H	106.8	6.45 1H	105.9	6.18 1H	106.9
6		164.4		165.4		164.3
7	6.22 1H	100.9	6.50 1H	99.4	6.26 1H	101.6
8		163.3		163.3		164.1
8a		100.3		101.5		102.4
1′		128.6		128.5		130.2
2′, 6′	7.31 2H	128.0	7.32 2H	128.2	7.32 2H	127.6
3′, 5′	6.80 2H	115.1	6.80 2H	115.2	6.88 2H	114.0
4′		157.6		157.7		159.8
6-*O*CH$_3$			3.82 3H	55.7		
4′-*O*CH$_3$					3.77 3H	55.4

[a] Proton NMR coupling patterns were omitted because they are identical for all compounds in the table.
[b] Measured in DMSO-d_6.
[c] Measured in DMSO-d_6.
[d] Measured in $CDCl_3$.

between the two subunits were detectable in any of the employed solvents. This finding is however in-line with earlier observations on simple dihydroisocoumarins where correlations from H-3 to C-1 are also usually weak or not observed at all.[2,5] Therefore, all possible linkage options had to be taken into account, provided that they were in-line with the established molecular mass of the molecule.

Shift values for the proton geminal to the oxygen of the alcohol moiety in related compounds (H-3 in the usually applied numbering system for dihydroisocoumarins) differ significantly in shift depending on whether or not the oxygen is free ($\delta_H \approx 4.8$ ppm),[6] esterified ($\delta_H \approx 5.8$ ppm),[6] lactonized ($\delta_H \approx 5.5$ ppm),[2] part of an ether ($\delta_H \approx 4.2$ ppm),[6,7] or phenyl ether bond ($\delta_H \approx 5.4$ ppm).[8] As the signals assignable to the two respective protons of compound **1** feature shift values which are both very similar and in the lower field part of the expected range ($\delta_{H\text{-}3} = 5.513$ ppm and $\delta_{H\text{-}3''} = 5.531$ ppm), all linkage options including one or two free alcoholic hydroxyl groups or an ether linkage between the two alcoholic moieties can be excluded. Moreover, the close similarity in shift implies that the two hydroxyl groups have a similar or identical substitution pattern.

Taking these considerations into account, only very few of the many potentially feasible linkage options of the two dihydroisocoumarin units remain: I.) Mixed ether linkage from one of the two alcoholic moieties to a free phenolic hydroxyl group of the other dihydroisocoumarin unit and δ-lactone formation within this second part of the molecule. There are four different options of this kind. II.) Ester linkage from the carboxyl group of one subunit to the alcoholic moiety of the other subunit and a mixed ether linkage between the remaining alcoholic moiety with any of the two free phenolic hydroxyl groups of the other subunit. There are also four theoretically possible linkage options of this kind. III.) Reciprocal esterification of the carboxyl group of both subunits with the alcoholic moiety of the respective other subunit (there is only one option for this kind of linkage and this is displayed as compound **1** in Fig. 1).

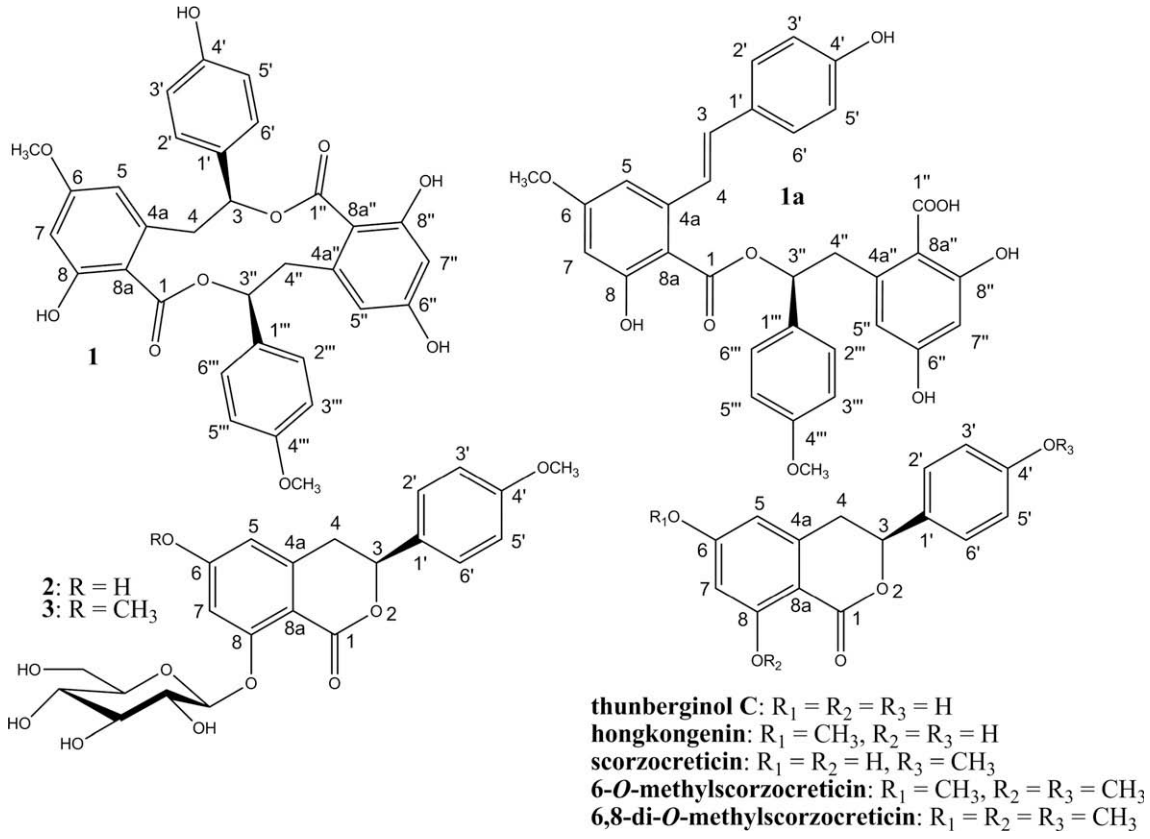

Figure 1. Structure of tragoponol (**1**), other dihydroisocoumarins (**2**–**4**), and bibenzyl (**5**) from *Tragopogon porrifolius*. Compound numbering of compounds **1** and **1a** is arbitrary and in analogy to the system used for monomeric dihydroisocoumarins **2**–**3**.

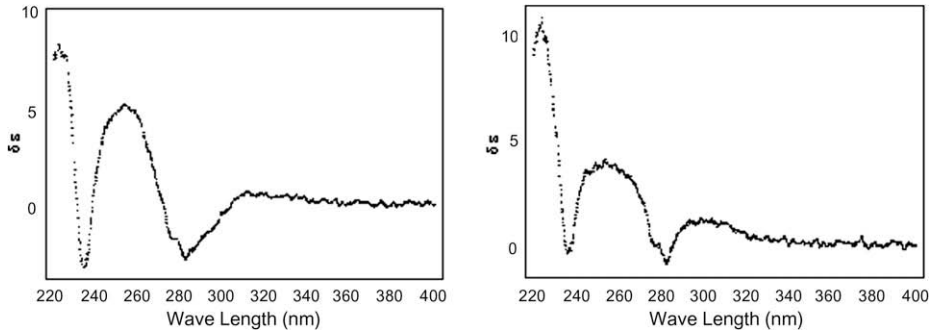

Figure 2. CD spectra from methylscorzocreticoside **3** and tragoponol **1** measured in MeOH.

Both options I and II comprise a mixed ether linkage between an alcoholic hydroxyl and a phenolic hydroxyl and thus an alkylation of the hydroxyl of the respective aromatic system.

The aromatic systems of comparable alkyl ethers versus their non-substituted parent compounds show the following shift differences (compare also Table 2)[4,5,9]: A slight ($\Delta\ \delta_C \approx +2$ ppm) downfield shift of the signal assignable to the affected aromatic carbon and a weak ($\Delta\delta_C \approx -1$ ppm) upfield shift of the signals assignable to the carbons vicinal to this carbon. Moreover, the signals assignable to the protons vicinal to the ether are shifted downfield by 0.1 to 0.2 ppm.

When comparing the signals assignable to the aromatic parts of the two stilbenoid moieties with the NMR data of the parent molecules hongkongenin and scorzocreticin (Table 2) no significant shift differences ($\delta_C < 2.0$ ppm and $\delta_H < 0.10$ ppm for all relevant signals) were observed. Thus, any substitution of the free phenolic hydroxyl groups can be ruled out and compound **1** was indirectly established as (7*S*,15*S*)-2,4,12-trihydroxy-7-(4-hydroxyphenyl)-10-methoxy-15-(4-methoxyphenyl)-7,8,15,16-tetrahydrodibenzo-[*c,i*][1,7]dioxacyclododecine-5,13-dione. We propose the trivial name tragoponol for this interesting new natural product, which is the first known dimeric dihydroisocoumarin. To the best of our knowledge tragoponol **1** is the first natural compound featuring a 7,8,15,16-tetrahydrodibenzo[c,i][1,7]dioxacyclododecine-5,13-dione ring system. However, 1,7-dioxacyclododecane-2,8-dione is known as a synthetic compound synthesized as a dimer from δ-valerolactone.[10] The most similar known natural product is lisianthioside, a dimeric secoiridoid glycoside from *Lisianthius jefensis* A.Robyns & T.S.Elias (Gentianaceae).[11]

As described above, many solvents were tried to more directly prove the structure established above. In the course of these investigations a degradation of compound **1** to compound **1a** was observed. First only traces of the degradation product with a double bond in the side chain of the hongkongenin sub-moiety were observed (Table 1). In a final measurement of the compound in DMSO-d_6 none of the original compound was left and the spectrum

Table 3
NMR data of the degradation product of tragoponol **1a**[*]

Position	Degraded 6-*O*-methyldihydroisocoumarin moiety				Position	4′-*O*-Methyldihydroisocoumarin moiety			
	^{1}H NMR[a]	^{1}H NMR[b]	^{13}C NMR[a]	^{13}C NMR[b]		^{1}H NMR[a]	^{1}H NMR[b]	^{13}C NMR[a]	^{13}C NMR[b]
01			n.d.	169.09	01″			n.d.	n.d.
03	6.787 1H, d (16.0)	6.696 1H, d (16.0)	129.44	126.73	03″	5.531 1H, m[*]	5.613 1H, dd (12.0, 2.5)	81.61	79.25
04	8.211 1H, d (16.0)	8.512 1H, d (16.0)	128.93	128.48	04″	3.264 1H, m[*]	3.285 1H, dd (16.5, 12.0)	35.67	33.47
						3.041 1H, m[*]	3.052 1H, dd (16.5, 2.5)		
04a			143.99	141.34	04a″			143.41	142.08
05	6.612 1H, br s	6.371 1H, d (2.0)	104.59	100.49	05″	6.264 1H, br s	6.286 1H, br s	107.88	106.80
06			163.04	160.24	06″			166.50	164.79
07	6.274 1H, br s	6.078 1H, d (2.0)	100.39	99.62	07″	6.226 1H, d (2.0)	6.207 1H, br s	102.15	100.80
08			164.87	166.63	08″			165.62	163.35
08a			111.76	111.03	08a″			101.33	100.27
01′			131.24	129.24	01‴			131.86	130.41
02′	7.386 1H, d (8.5)	7.304 1H, d (8.5)	128.81	127.43	02‴	7.421 1H, d (8.5)	7.437 1H, d (8.5)	128.73	127.96
03′	6.743 1H, d (8.5)	6.743 1H, d (8.5)	116.09	115.20	03‴	6.966 1H, d (8.5)	6.980 1H, d (8.5)	114.78	113.70
04′			157.88	156.46	04‴			161.36	159.25
05′	6.743 1H, d (8.5)	6.743 1H, d (8.5)	116.09	115.20	05‴	6.966 1H, d (8.5)	6.980 1H, d (8.5)	114.78	113.70
06′	7.386 1H, d (8.5)	7.304 1H, d (8.5)	128.81	127.43	06‴	7.421 1H, d (8.5)	7.437 1H, d (8.5)	128.73	127.96
6-OCH_3	3.802 3H, s	3.705 3H, s	55.31	54.37	4‴-OCH_3	3.813 3H, br s	3.770 3H, s	55.50	54.93

[*] Measured at 800 and 200 MHz, respectively.
[a] Measured in MeOH-d_4, at 3.31 ppm (^{1}H NMR) and 49.00 ppm (^{13}C NMR), respectively.
[b] Measured in DMSO-d_6, and referenced to solvent residual and solvent signals at 2.50 ppm (^{1}H NMR) and 39.50 ppm (^{13}C NMR), respectively.

only showed signals assignable to the degradation product **1a**, (*S*,*E*)-2,4-dihydroxy-6-(2-(2-hydroxy-6-(4-hydroxystyryl)-4-methoxybenzoyloxy)-2-(4-methoxyphenyl)ethyl)benzoic acid. NMR data of this compound measured in MeOH-d_4 and DMSO-d_6 are displayed in Table 3.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.01.016.

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