Chemical Constituents of *Santolina chamaecyparissus*

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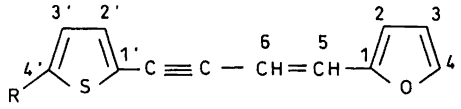
Lam, J., Bildsøe, H., Christensen, L. P., and Thomasen, T., 1989. Chemical Constituents of *Santolina chamaecyparissus*. – Acta Chem. Scand. 43: 799–802.

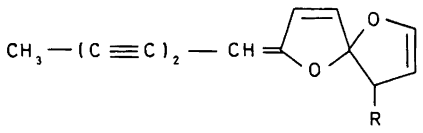
Roots of *Santolina chamaecyparissus* contain, besides a number of previously reported acetylenes, an acetylenic isovaleric acid ester known from *Santolina rosmarinifolia*. The aerial parts of *Santolina chamaecyparissus* contain a series of acetylenes known from the root material and four spiroketalenol ethers, known from *Santolina rosmarinifolia*. Furthermore, two previously suggested precursors (*Z*- and *E*-isomers) of several thiophene–furan acetylenes occur in the roots and aerial parts of the plant. The possible presence of a labile disulfur compound is discussed.

Santolina chamaecyparissus L. (Anthemideae) grows wild in North Africa (Tunisia and Morocco) and near the Mediteranean coasts of Southern Europe. Buolos[1] mentions the plant in his book dealing with medicinal plants of North Africa as a remedy in folk medicine against intestinal worms, as a spasmolyticum, or for other purposes. The plant contains a series of mono- and sesqui-terpenes. One of the abundant monoterpenes is artemisiaketone (**1**), $(CH_3)_2C{=}CHCOC(CH_3)_2CH{=}CH_2$, known from another plant *Artemisia annua*[2,3] of the same tribe of Asteraceae. Somewhat confusedly, the name artemisiaketone has also been applied to another compound, $CH_3(C{\equiv}C)_3CH{=}CH(CH_2)_2COCH_2CH_3$, which occurs in e.g. *Artemisia vulgaris*,[4,5] also from the tribe Anthemideae.

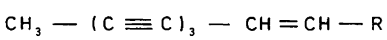
S. chamaecyparissus, propagated in a greenhouse of the University of Aarhus, contains in its roots a number of acetylenic compounds (**2a**, **3a–6**) previously identified by Bohlmann *et al.*[6,7]

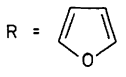


R = H, *Z* isomer: **2a**
R = H, *E* isomer: **2b**
R = CHO, *Z* isomer: **3a**
R = CHO, *E* isomer: **3b**
R = CH_2OH, *Z* isomer: **4a**
R = CH_2OH, *E* isomer: **4b**
R = CH_2OAc, *Z* isomer: **5a**
R = CH_2OAc, *E* isomer: **5b**
R = $CH_2OOCCH_2CH(CH_3)_2$, *Z* isomer: **10a**
R = $CH_2OOCCH_2CH(CH_3)_2$, *E* isomer: **10b**

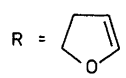


R = OH, *E* isomer: **6**
R = H, *Z* isomer: **11a**
R = H, *E* isomer: **11b**
R = OAc, *Z* isomer: **12a**
R = OAc, *E* isomer: **12b**





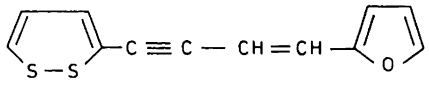
Z isomer: **8a**
E isomer: **8b**



Z isomer: **9a**
E isomer: **9b**

Results and discussion

S. chamaecyparissus has been repeatedly investigated in our laboratories for its acetylene content (unpublished data), both in the roots and in the aerial parts, from which also artemisiaketone (**1**) has been isolated (Table 1). A red-coloured fraction from the root extract had an absorption spectrum with a broad maximum at about 540–545 nm, somewhat more red-shifted than the spectrum of the thiarubrines known from *Eriophyllum caespitosum*, *E. lanatum* and other genera of the family Asteraceae.[8,9] This suggests that a biological reaction may occur similar to that responsible for the formation of thiarubrines in other plants of Asteraceae. The red compound is present only in small amounts, and we have not been able to identify it, but we suggest that it is related to **2a** and **2b** based on its absorption spectrum. Its polarity (higher than that of **2a** and **2b**) and its mass spectral pattern are properties which would fit the structure **7**.



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Such a compound is likely to be very unstable and will easily extrude one sulfur atom. The mass spectrum would then be that of **2a** and **2b** as is indeed the case for the red fraction when it is subjected either to direct inlet mass spectrometry or to GLC/MS studies. This fraction contains, besides the red compound, **3a**, **3b** and **5a**, **5b** which exhibit fragmentation patterns different from those of **2a** and **2b**. Compound **2a**, **2b** and the red compound show peaks at *m/z* 200 of high relative intensity, whereas neither **3a**, **3b** nor **5a**, **5b** exhibit any important fragment at *m/z* 200.

A GLC/MS examination of a crude extract from root material of *S. chamaecyparissus* on a 20 m OV-101 capillary column revealed the presence of **2a** and **2b** appearing at 29 and 31 min. A fragmentation pattern corresponding too that of **2a** and **2b** is seen ca. 3 min later, signaling the presence of a compound that after extrusion of one sulfur atom exhibits the same mass fragmentation pattern. The direct inlet mass spectra of thiarubrines (dithiacyclohexadienes) isolated in this laboratory from *E. lanatum* show very intense ions at *m/z* 228 (the M^+) and a minor fragment at *m/z* 196 (M^+-S) (for MS data see also Ref. 9). GLC/MS studies showed no sign of the molecular ion of thiarubrines. It may hence be expected that a disulfur compound of *S. chamaecyparissus* such as **7**, if present, would extrude a sulfur atom during the passage through the capillary column. The direct inlet mass spectrum of the red *S. chamaecyparissus* fraction did not give any sign of the presence of a compound with M^+ 232. During GLC/MS studies, a mass fragmentation pattern corresponding to that of **2a**, **2b** supplemented with a signal at *m/z* 232, was seen only after injection onto short capillary columns (see the Experimental).

The presence of **2a** and **2b** was verified by the separation of a fraction from the root material of *S. chamaecyparissus* by HPLC (see the Experimental and Fig. 1). The ^1H NMR spectra were in good agreement with the structures of these compounds (see the Experimental). An additional peak (Fig. 1) reveals the presence of minute amounts of **8a** and **8b** identified by the UV spectrum (Fig. 2) and GLC/MS. The mass spectrum as well as the UV spectrum agree well with the data of an authentic specimen previously produced in our laboratory (unpublished data) and with data from literature.[10] In addition we isolated and identified compounds **9a**, **9b**, **10a**, **10b** and **12a**, **12b** from the roots. Compounds **9a**, **9b** have previously been isolated from root material of *Chrysanthemum leucanthemum*[10,11] and **10a**, **10b** from *S. rosmarinifolia* root material.[12]

From the aerial parts, a weakly polar fraction, studied by GLC/MS, seemed to contain two compounds of molecular weight 182, very likely **9a** and **9b**. Two other compounds, of molecular weight 180, corresponded well in their MS pat-

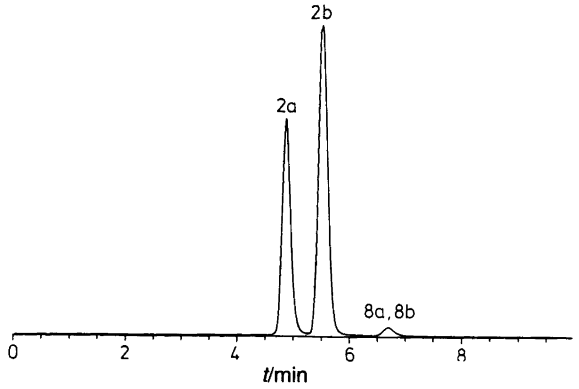


Fig. 1. Separation of **2a** (*Z*-isomer) and **2b** (*E*-isomer) by HPLC revealed the presence of a minute amount of material which was identified as the precursors of **2a** and **2b**.

Table 1. Compounds isolated from roots and leaves/stems of *Santolina chamaecyparissus* in mg kg^{-1}.

Compound		Roots	Leaves/stems
1			920
2a	(*Z*)	432	see text
2b	(*E*)	294	
3a	(*Z*)	6	<0.1
3b	(*E*)	4	<0.1
4a	(*Z*)	17.5	–
4b	(*E*)	5.4	–
5a	(*Z*)	118	<0.1
5b	(*E*)	40	<0.1
6	(*E*)	37	102
7		<0.1	–
8a	(*Z*)	0.6	see text
8b	(*E*)	2.5	
9a	(*Z*)	<0.5	<0.1
9b	(*E*)	<0.5	<0.1
10a	(*Z*)	15.5	–
10b	(*E*)	10.5	–
11a	(*Z*)	–	96
11b	(*E*)	–	580
12a	(*Z*)	120	see text
12b	(*E*)	30	

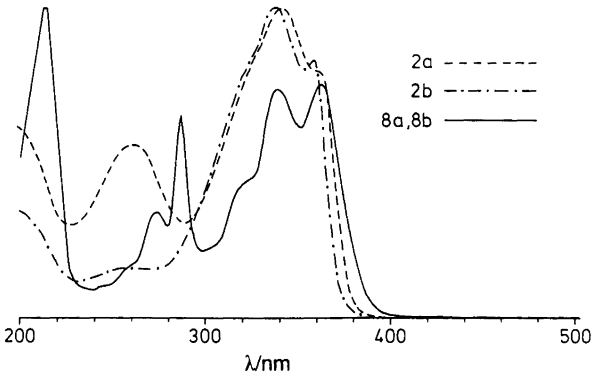


Fig. 2. Ultraviolet spectra of **2a**, **2b** and **8a**, **8b**.

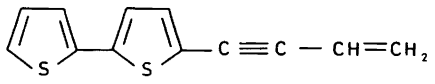
terns with those of **8a** and **8b**. Moreover, **2a** and **2b** were proved to be present.

Thus the suggested precursors of **2a** and **2b**[6,7] are present in all parts of this plant. The fraction containing **2a**, **2b** and **8a**, **8b** exhibits a UV spectrum of all four compounds. The composite spectrum is not usable for the determination of the exact amounts of **2a**, **2b** and **8a**, **8b** present in the aerial parts of *S. chamaecyparissus*. In a previous study a total amount of 3.8 mg kg^{-1} of the four compounds was found, with **2a** and **2b** being greatly predominant. In addition, the aerial parts contain minute amounts of **3a**, **3b**, **5a**, **5b**, **9a**, **9b** and **12a**, **12b** (GLC/MS, see Table 1).

From the following fractions of the aerial parts of the spiroketalenol ethers (**11a**, **11b**) were isolated and characterized. They are well known from other genera of Anthemideae, e.g. *S. rosmarinifolia*[12] and *C. leucanthemum*,[11] in which they occur in the root material together with **12a** and **12b**.

The *S. chamaecyparissus* plants were propagated in sandy soil. The soil was extracted with diethyl ether and **2a** and **2b** were isolated from the extract in minute amounts. In addition, a compound of *M* 256, probably a spiroketalenol ether, was also detected. The thiophene–furan acetylenes exuded from the roots may play a defense role against attack of the plant from nematodes or microorganisms.

We have previously observed such an exudate in the soil around *Echinops sphaerocephalus* where compounds α-terthienyl, **13** and **14** were present in the soil in amounts proportionally different from those in the root material, where especially the chlorine compound (**14**) occurs in a considerably higher relative amount. This indicates that



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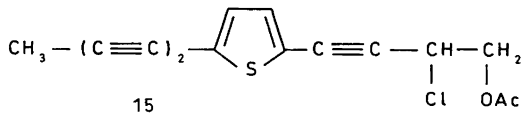

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exudation into the soil had occurred rather than that extraction from root material detached from the plant in the soil had been performed. Compounds containing thiophene rings from *Echinops* species are known to act biologically more effectively in UV light than in the dark,[13] although α-terthienyl is also biologically active in the dark[14] and thus biological defence of the plants after exudation into the surroundings is possible.

Experimental

Root material (1200 g) was extracted with Et_2O and from the extract a number of fractions were obtained by flash chromatography (silica gel; light petroleum–Et_2O gradient).[15]

The weakly polar fractions containing **2a**, **2b** and **8a**, **8b** were eluted at much lower polarity than the unknown red compound, which was extremely unstable. The red fraction was repeatedly subjected to column chromatography and preparative TLC and it has a polarity corresponding to those of **3a**, **3b** and **5a**, **5b** (GLC/MS). Aerial parts (1600 g, leaves and stems) were extracted with light petroleum (b.p. <50 °C).

The GLC/MS studied were carried out on a VG Trio 2 GC/MS/DS mass spectrometer combined with a Hewlett–Packard gas chromatograph (HP 5890). The fractions were usually separated on a 20 m OV-101 capillary column and in special cases, when very unstable compounds were to be separated, on a shorter capillary column (OV-101) of 3 or 5 m. The column temperature was usually 70 °C for about 4 min and then increased by 5 °C min^{-1} until 250 °C was reached. For 3 or 5 m column separations the starting temperature was 40 °C.

In one case, where **2a** and **2b** had only been separated analytically by GLC, separation by HPLC on an RP-18 reversed-phase column was carried out eluting with CH_3CN–H_2O (70:30) and by repeated injection onto an HP 1090M HPLC system from Hewlett–Packard.*

MS and UV spectra were recorded for all the compounds and ^1H NMR (200 MHz, $CDCl_3$, TMS int. standard) were run for **1–6** and **9a–12b**.

The amounts of the *Z*- and *E*-isomers were determined by ^1H NMR and UV spectroscopy.

^1H NMR (**2a**): δ 7.04 (1 H, br d, *J* 3.5 Hz, H-2), 6.47 (1 H, ddd, *J* 3.5, 1.8, and 0.6 Hz, H-3), 7.42 (1 H, dt, *J* 1.8, 0.7 and 0.5 Hz, H-4), 6.62 (1 H, dt, *J* 11.8, 0.6 and 0.6 Hz, H-5), 5.72 (1 H, br d, *J* 11.8 Hz, H-6), 7.25 (1 H, dd, *J* 3.7 and 1.2 Hz, H-2′), 7.00 (1 H, dd, *J* 5.2 and 3.7 Hz, H-3′), 7.30 (1 H, dd, *J* 5.2 and 1.2 Hz, H-4′). For a previously recorded ^1H NMR spectrum of **2a** see also Ref. 6.

^1H NMR (**2b**): δ 6.34 (1 H, dm, *J* 3.4 Hz, H-2), 6.40 (1 H, dd, *J* 3.4 and 1.8 Hz, H-3), 7.38 (1 H, dm, *J* 1.8 Hz, H-4), 6.75 (1 H, br d, *J* 16.0 Hz, H-5), 6.25 (1 H, br d, *J* 16.0 Hz, H-6), 7.19 (1 H, dd, *J* 3.7 and 1.2 Hz, H-2′), 6.97 (1 H, dd, *J* 5.2 and 3.7 Hz, H-3′), 7.25 (1 H, dd, *J* 5.2 and 1.2 Hz, H-4′).

Acknowledgements. The use of the MS and NMR instruments at the University of Aarhus, sponsored by the Danish Research Council (SNF), the Carlsberg Foundation and Aarhus University Research Foundation, is acknowledged.

*As it is likely that some of the isomers ratios are affected by UV transformation of one isomer in to the other or by seasonal variation strict separation of the *Z*- and *E*-isomers is generally not very informative.

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Received March 8, 1989.