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## Note

### High-performance liquid chromatography of *cis*- and *trans*-en-in-di-cyclo ethers (spiro ethers) in *Matricaria chamomilla* L. flowers and in chamomile extracts

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The *cis*- and *trans*-en-in-dicyclo ether 2-hexa-2,4-diin-1-ylidene-1,6-dioxaspiro[4,4]non-3-ene, (1 and 2; Fig. 1), have been isolated from some species of *Matricaria*<sup>1</sup> and some preliminary pharmacological investigations have been performed<sup>2–4</sup>. Apparently, only the *cis* isomer exerts a spasmolytic action, which is at least ten times higher than that of papaverine<sup>3,8</sup>. The separation of (1) and (2) in chamomile has been accomplished by thin-layer chromatography<sup>5</sup>, gas-liquid chromatography (GLC)<sup>6</sup> and liquid chromatography on Kieselgel columns<sup>7</sup>.

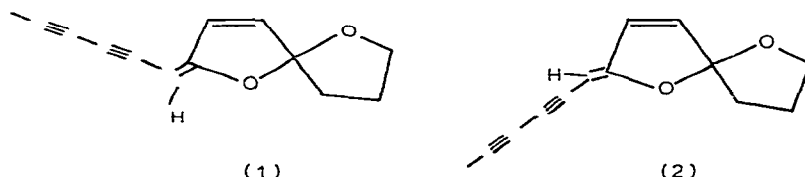


Fig. 1. Structures of the spiro ethers. 1 = *cis*-En-in-dicyclo ether; 2 = *trans*-en-in-dicyclo ether.

High-performance liquid chromatography (HPLC) seemed to be the method of choice for the analysis of these compounds, owing to the simple preparation of the sample and the mild conditions of operation. After preliminary runs, we found that on a reversed-phase column an excellent separation of (1) and (2) is possible, and detection at 317 nm ensures high sensitivity owing to the high absorbance maximum ( $\epsilon = 19,500 \text{ l mol}^{-1} \text{ cm}^{-1}$ ).

## EXPERIMENTAL AND RESULTS

Dicyclo ethers are present almost exclusively in the receptacle of the inflorescence, where (–)- $\alpha$ -bisabolol and its oxides are not found. Pure (1) and (2) have been prepared by extracting receptacles of *Matricaria chamomilla* L. flowers with dichloromethane and separating them on a silica gel column (*n*-hexane-ethyl acetate, 97:3). GLC analysis showed that the (1) and (2) were 99.1% and 98.1% pure, respectively.

Routine analysis of the flowers is performed by stirring in methanol (10 ml) 1 g of pulverized flowers (2 h, room temperature) and injecting 5  $\mu$ l of the solution after filtration. Analysis of water-alcohol extracts is performed by direct injection of 5  $\mu$ l of the extract. A Perkin-Elmer Series 3 liquid chromatograph equipped with a microprocessor-controlled pump module, a Rheodyne injector, a Model 023 recorder, a Model 55B UV-visible detector and a 25  $\times$  0.26 cm I.D. HC-ODS Sil-X reversed-phase column (Perkin Elmer) was used. A Minigrator integrator (Spectra-Physics, Santa Clara, CA, U.S.A.) was employed. The operating conditions for HPLC were as follows: room temperature; eluting solvent, acetonitrile-water containing 2% of acetic acid (2:3); flow-rate, 1 ml/min; wavelength of UV detector, 317 nm; recorder chart speed, 1 cm/min; sample size, 5  $\mu$ l; peak width parameter value of integrator, 5; slope sensitivity, 250.

The quantitative determination of spiro ethers in unknown samples under the above conditions was studied using external standardization, and excellent precision and reliability were obtained. The spiro ethers were well separated, giving retention times ( $t_R - t_0$ ) of 3.2 min (*trans*-en-in-dicyclo ether) and 5.2 min (*cis*-en-in-dicyclo ether) (Fig. 2).

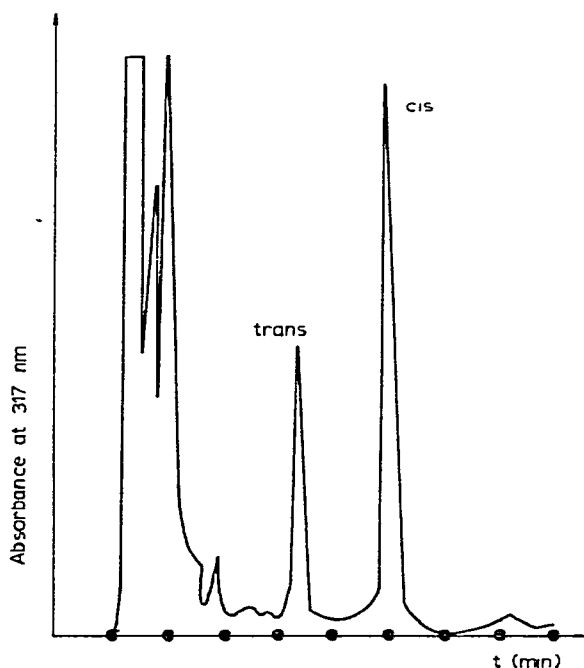


Fig. 2. High-performance liquid chromatogram of a chamomile extract.

## DISCUSSION

Previous work has shown that HPLC is an excellent method for the determination of flavonoids<sup>8,9</sup> and coumarins<sup>10</sup> in *Matricaria chamomilla* L. flowers and chamomile extracts. The method has also been applied satisfactorily to dicyclo ethers

as a result of its simplicity, sensitivity and mildness of conditions. Analyses were carried out at room temperature, thus preventing possible *cis-trans* isomerization at elevated temperature, and the contents of (1) and (2) in chamomile extracts were determined. However, a systematic study of the stability of dicyclo ethers is required as the available data provide no information on this aspect. Finally, it should be possible to apply the method to the determination of dicyclo ethers and other components in chamomile infusions.

#### REFERENCES

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