Comparative Study of *Tanacetum* Species Growing in Bulgaria

Milka N. Todorova and Ljuba N. Evstatieva

* Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria. E-mail: toodorova@orgchem.bas.bg

** Institute of Botany, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

* Author for correspondence and reprint requests


*Tanacetum* species, Asteraceae, Sesquiterpene lactones

Chemical investigation of the Bulgarian species *Tanacetum parthenium*, *T. millefolium*, *T. achilleifolium*, *T. corymbosum* and *T. macrophyllum* afforded in addition to 24 known sesquiterpene lactones a new 12,8-eudesmanolide 22. Besides, four known highly oxygenated terpenoids and a new keto-diol 30 of the rare iphionane skeleton were also isolated. The structures were elucidated on the basis of their spectral properties. The accumulation of the different sesquiterpene lactones in the studied species and their classification is discussed.

Introduction

The genus *Tanacetum* is one of the largest genera in Asteraceae family comprising 14 species distributed in Europe (Heywood, 1976). However, its exact position is not yet determined as it is considered to be quite heterogeneous regarding the botanical classification. Thus, some of the *Tanacetum* species are believed to belong to the genus *Chrysanthemeum*, while others are classified under the genus *Pyrethrum*. The taxonomical rank of some *Tanacetum* species is also under discussion. Thus, Heywood described *T. millefolium* and *T. achilleifolium* as two different species although they are not always clearly separated (Heywood, 1976), while Stojanov et al. based on the close morphological grounds treats them as varieties of *T. millefolium* – var. *millefolium* and var. *achilleifolium* (Stojanov et al., 1967). The chemical composition of *Tanacetum* species have received much attention because the terpenoids, in particular the sesquiterpene lactones, are believed to play a substantial role in the plant taxonomy. Moreover, most of them have shown to possess different biological activities (Abad et al., 1995).

The *Tanacetum* genus is represented in Bulgaria by six species (Andreev et al., 1992), of which *T. vulgare* L. and *T. macrophyllum* (Waldst. & Kt.) Schultz Bip. have been previously studied by us (Todorova and Ognyanov, 1985; Todorova and Ognyanov, 1999; Ognyanov and Todorova, 1983). Continuing the chemical investigation of Bulgarian medicinal plants of the Asteraceae family we extended our examination on the other four species – *T. achilleifolium* (Bieb.) Schultz Bip., *T. millefolium* (L) Tzvelev, *T. corymbosum* (L) Schultz Bip., *T. parthenium* (L) Schultz Bip. The literature search revealed that *T. parthenium* had been subject of many scientific papers reviewed by Milbrodt et al. (1997) and Knight et al. (1995). However, no work had been reported so far on *T. millefolium* and *T. achilleifolium*. Very recently the study appeared on *T. corymbosum* (Milosavljevic et al., 1999).

The present study focuses on the sesquiterpene lactone profile of the Bulgarian *Tanacetum* species.

Experimental

Plant material

The aerial parts of the studied samples were collected at flowering stage in July, 1999 from the following locations: Black Sea coast near Kavarna (*T. achilleifolium* and *T. millefolium*) and Losenska mountain (*T. corymbosum*, *T. macrophyllum* and *T. parthenium*). Voucher specimens (L. 9931 – 9935, respectively) were deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia.

Extraction and isolation

The air-dried leaves and flowers of each sample (the amounts are given below) were extracted exhaustively with CHCl₃ (3 x 600 ml) at room temp-
perature. After evaporation of the solvent under reduced pressure the resulting extracts were worked up as described (Todorova and Ognyanov, 1985). The crude lactone fractions (see below) were separated by column chromatography (CC) on silica gel, using solvent mixtures (n-hexane / acetone) with increasing polarity. The fractions containing lactones (IR control) were further subjected to repeated CC and/or prep. TLC (silica gel) to yield the individual components. The known compounds were identified by spectroscopic methods and TLC comparison using authentic samples as references.

**T. parthenium** (340 g) afforded 5.0 g lactone fraction which yielded: 1 (24 mg), 2 (6 mg), 3 (5 mg), 4 (4 mg), 5 (5 mg), 6 (6 mg), 7 (15 mg), 8 (11 mg), 9 (4 mg), 10 (3 mg), 11 (4 mg), 12 (6 mg), 13 (5 mg), 14 (3 mg) and 15 (7 mg).

**T. millefolium** (240 g) afforded 2.1 g lactone fraction which yielded: 16 (160 mg), 17 (4 mg), 18 (15 mg), 19 (15 mg) and 20 (12 mg).

**T. corymbosum** (134 g) afforded 2.2 g lactone fraction which yielded: 21 (8 mg) and 22 (10 mg).

1ß,4a-Dihydroxy-6a-tigloyloxy-11 (13)-eudesmen-12,8-olide (22)

Colourless oil, CIMS (APCI) m/z (rel. int.) 365 [M+1]+ (8), 347 [M+1=18]+ (42), 265 [M+1=100]+ (48), 247 [347=100]+ (100), 229 [247=18]+ (93), 182 (52). 1H NMR (250 MHz, in CDCl3) δ: 3.49 (1H, dd, 4.0, 9.7, H-1), 1.93 (1H, d, 10.8, H-5), 4.05 (1H, ddd, 3.9, 11.8, 11.8, H-8), 2.87 (1H, br. t., 11.8, H-7), 5.79 (1H, dd, 10.8, 11.8, H-6), 5.25 (1H, d, 2.9, H-13), 6.07 (1H, d, 3.1, H-13'), 2.54 (1H, dd, 3.8, 11.8, H-9), 1.48 (1H, t, 11.8, H-9'), 1.60–1.80 (4H, m, 2H-2 and 2H-3), 1.05 (3H, s, H-14), 1.26 (3H, s, H-15), 6.95 (1H, qq, 1.3, 7.0, H-3'), 1.83 (3H, dq, 7.0, 1.3, H-4'), 1.88 (3H, dq, 1.3, 1.3, H-5').

**T. macrophyllum** (117 g) afforded 2.0 g lactone fraction which yielded: 2 (5 mg), 3 (3 mg), 5 (4 mg), 6 (6 mg), 23 (9 mg), 24 (2 mg), 25 (7 mg), 26 (3 mg), 27 (7 mg), 28 (12 mg), 29 (11 mg), 30 (8 mg).

5ß,11-Dihydroxy-iphionan-4-one (30)

White powder; EIMS (70 eV) m/z (rel. int.) 254 [M]+ (1). 236 [M-18]+ (3), 221 [236-15]+ (8), 203 [221-18]+ (10.5), 195 [M-(CH3)2COH]+ (8), 178 [221-CH3CO]+ (67.7), 170 (95.2), 152 (92.7), 135 (87.9), 109 (74.2), 97 (30.6), 95 (28.2), 93 (29.8), 81 (41.1), 71 (38.7), 59 (61.3), 55 (33.3), 43 (100); 1H NMR (250 MHz, in CDCl3) δ: 1.00 (s, 3H, H-14), 1.18 (s, 6H, H-12 and H-13), 2.18 (s, 3H, H-15), 3.35 (dd, 1H, H-3, J1=J2 = 10 Hz).

### Results and Discussion

Each of the plant samples was extracted with chloroform and the fractions enriched in sesquiterpene lactones were worked up as described in the experimental section to give the individual components. Their identification and structure elucidation were achieved using spectral methods.

**T. parthenium** afforded fifteen sesquiterpene lactones, including the single germacranolide parthenolide (1) (Govindachari et al., 1964) which was the major one. The latter, as well as canin (2) artecanin (3), tanapartin-ß--peroxide (4), secovanolide B (9) (Samek et al., 1973) are known constituents for *T. parthenium*. However, the eudesmanolides armefolin (10) (Mata et al., 1984) and 1ß-hydroxyarbusculin A (11) (Samek et al., 1973) were found for the first time in this species. Furthermore, the guaianolides 8-deoxycumambrin B (12) (Sosa et al., 1989), achillin (13) (Marco et al., 1988), 4a,10a-dihydroxy-1,5H-guaia-2,11(13)dien-12,6a-olide (14) (Zdero et al., 1987) and 1ß-hydroxycichopumelide (15) (El-Masry et al., 1984) have not been reported so far as constituents of any *Tanacetum* species.

This is the first study of *T. achilleifolium* and *T. millefolium*. Surprisingly, the lactone fractions of these two species proved to be practically identical regarding the main components which were identified as the eudesmanolides vulgarin (16) (Ohno et al., 1980), 4-epi-vulgarin (17) (González et al., 1983) and 11,13-dihydro-santamarine (18) (Shafizadeh et al., 1971). Among them, the lactone 16 was the principal constituent. In addition, two davanone derivatives 19 and 20 (Appendino et al., 1984) were also isolated.

**T. corymbosum** of Serbian origin is reported to be free of sesquiterpene lactones (Milosavljevic et al., 1999). However, two closely related lactones...
21 and 22 were isolated from this species of Bulgarian origin. Whereas the eudesmanolide $1\beta,4\alpha,6\alpha$-trihydroxy-11(13)-eudesmen-12,8-olide (21) had been found previously in *T. densum* (Gören *et al.*, 1993), the lactone 22 proved to be a new natural product. Its structure followed easily from the MS and $^1$H NMR spectra which revealed that the only difference in 21 and 22 is the presence of an ester side chain in 22. The latter was identified as a tigloyloxy group the position of which at C-6 followed from the downfield shift of H-6 signal and its vicinal interactions with H-5 and H-7. COSY experiments allowed the assignment of all the proton signals. The relative stereochemistry at the chiral centres was derived from the observed spectral data and NOE measurements. Thus, the lactone no. 22 was $1\beta,4\alpha$-dihydroxy-$6\alpha$-tigloyloxy-11(13)-eudesmen-12,8-olide.
The repeated examination of *T. macrophyllum* yielded, in addition to the previously isolated lactones 2, 3, 23–26 (Todorova and Ognyanov, 1985), secotanapartholides – A (5) and -B (6) (Bohlmann et al., 1982) and artecalin (27) (Geissman et al., 1969). Whereas the lactones 5 and 6 are known constituents of *Tanacetum* species, artecalin (27) has not been found so far in the genus *Tanacetum*. Furthermore, three non-lactonic terpenoids, 28–30 were also isolated which proved to be new for
the genus Tanacetum. Of them, the acyclic monoterpene diol 28 has been found in Cinnamomum camphora (Takaoka, 1976), and the secoeudesmane 29 has been isolated from Eriocephalus species (Asteraceae) (Zdero et al., 1987). The compound 30 had not been found before. Its structure was deduced by means of the following spectroscopic data. The EI mass spectrum was very informative showing besides the molecular peak \([M]^+\) at \(m/z\ 254\) consistent with a molecular formula \(C_{15}H_{26}O_3\), the fragments at \(m/z\ 236\ [M-H_2O]^+, 221\ [236-CH_3]^+, 218\ [236-H_2O]^+, 195\ [M-(CH_3)_2COH]^+\ and 178\ [221-CH_3CO]^+\), which revealed the presence of one OH group, one hydroxyisopropyl moiety and a methyl ketone group. The \(^1H\) NMR spectrum exhibited signals for four methyl groups – an angular methyl (\(\delta\ 1.00\)), two methylys of a hydroxyisopropyl group (\(\delta\ 1.18, s, 6H\)), and a methyl ketone (\(\delta\ 2.18\)). Further, a proton triplet is visible at \(\delta\ 3.32\), which was assigned to H-3. These data were accommodated by the carbocyclic iphonane skeleton, bearing a ketone group at C-4 and two hydroxyl groups at C-5 and C-11. Hence, the new terpenoid 30 was identified as 5β,11-dihydroxy-iphonan-4-one. The latter is a representative of the rarely occurring type of sesquiterpenoids in which the six-membered ring of an eudesmane skeleton has undergone ring contraction. The co-occurrence of 4,5-seco-eudesmane compounds and that with iphonane skeleton, reported also in other species of Asteraceae family (El-Ghazouly et al., 1987; Castilo et al., 1995; Jaku-}

The overall picture of the sesquiterpene lactones found in the Tanacetum species growing in Bulgaria is presented in Table I. The studied taxa are classified under two different sections according to the botanical characteristics. To a certain extent the results of our investigation are in agreement with this classification. Thus, T. achilleifolium and T. millefolium belonging to section Tanacetum produce one skeletal type of lactones only. Moreover, they are the only Bulgarian Tanacetum species producing 11,13-dihydroeudesmanolides, a fact which could be of chemotaxonomical interest. The other member of this section, T. vulgare was studied by us earlier, and the results revealed that this species is also producing lactones of one skeletal type only (Todorova and Ognyanov, 1999). Moreover, as can be seen in Table I., the existence of three chemotypes is demonstrated – germacranolide (sample T. vulgare 1), eudesmanolide (sample T. vulgare 2) and lactone free (sample T. vulgare 3). The identical chemical composition of T. achilleifolium and T. millefolium found by us could indicate the close relationship between these two taxa. Many intermediate forms observed as well as lack of any chemical evidence for the differentiation of these two species strongly support the classification of Stojanov et al. (1967) who described two varieties of A. millefolium – var. millefolium and var. achilleifolium.

| Table I. Sesquiterpene lactones found in the Bulgarian Tanacetum species. |
|-----------------------------------|----------------|-----------------|----------------|
| I. Sect. Tanacetum                |                |                |
| T. vulgare-1*                     | 31, 32, 33, 34 | 9, 10, 11, 18, 33, 34, 35, 36 |
| T. vulgare-2*                     | –              | 16, 17, 18     |
| T. vulgare-3*                     | –              | 16, 17, 18     |
| T. achilleifolium                 | –              |                 |
| T. millefolium                    | –              |                 |
| II. Sect. Pyrethrum               |                |                |
| T. corymbosum                     | –              | 21, 22          |
| T. macrophyllum                   | 26             | 2, 3, 4, 5, 6, 23, 24, 25 |
| T. parthenium                     | 1              | 8, 9, 10, 11    |

* Published data (Todorova and Ognyanov, 1999).
Ger – germacranolides; Gu – guaianolides; Eu – eudesmanolides.
Further, two of the species belonging to Sect. *Pyrethrum* (*T. parthenium* and *T. macrophyllum*) produce lactones of all the three skeletal types. Of them, the guaianolides are the most abundant and with a large variety of oxygen containing functional groups, although the germacranolide parthenolide (1) is the major lactone in *T. parthenium*. Surprisingly, the third member of this section, *T. corymbosum* contained only lactones of the considerably small group of eudesman-12,8-olides which are not typical for the genus *Tanacetum*.

Hence, the sesquiterpene lactone profile of the Bulgarian *Tanacetum* species studied is in agreement with the botanical classification with the only exception – *T. corymbosum*. This species, producing one skeletal type of lactones appears to be closer to the species of sect. *Tanacetum*. Moreover, similar to sample *T. vulgare* 3, a collection of *T. corymbosum* from Serbia was reported to be free of lactones. All these results could be an indication for reconsideration the position of *T. corymbosum*.

**Acknowledgements**

The authors are grateful for the financial support of Project X-911 provided by the Bulgarian National Research Foundation.


