ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 57, nr 1: 85-92
1988

Phytochemical studies of the herb, *Tragopogon orientalis* L. (*Asteraceae*). 1. The components of a petroleum ether extract

TADEUSZ KRZACZEK, HELENA DANUTA SMOLARZ

Department of Pharamaceutical Botany, Medical Academy, Staszica 4, 20-081 Lublin, Poland

(Received: December 23, 1986. Accepted: March 25, 1987)

Abstract

Hydrocarbons, aliphatic alcohols, triterpenes and sterols were shown to occur in the herb, *Tragopogon orientalis* L. The following were obtained in crystalline form: n-triacontan, hexacosanol, β-amyrin and lupeol. The presence of β-sitosterol, stigmasterol, cholesterol and four unidentified sterols was demonstrated by gas chromatography.

Key words: Tragopogon orientalis L., hydrocarbons, triterpenes, sterols

INTRODUCTION

Eastern goat's beard, *Tragopogon orientalis* L., is found over the entire territory of Poland (Kucowa 1972). It is a lactagogue and is considered to be a desired component of fodder for cows (Włodarczyk 1980, Rutkowska 1984). Schlechtendal et al. (1887) reported that the roots of *T. orientalis*, *T. porrifolius* and other species are eaten as a valued vegetable. Mowszowicz (1967) and Grynia (1974) however, consider *T. orientalis* to be a poisonous plant. Krzaczek et al. (1981) have demonstrated the rather high cytostatic activity of an ethanol extract of this herb.

The chemical composition of this plant is poorly known. Moldenhawer (1953) reports a 6% oil content in the fruits, and Kucerov and Siraeva (1980) have determined the percentage of fructose, sucrose and glucose in the nectar of *T. orientalis*. Kuzmanov et al. (1970) conducted chromatographic analysis of phenolic components and phenylpropane derivatives in 9 species of

the genus *Tragopogon* and suggested the presence of cinnamic and chlorogenic acids in *Tragopogon orientalis* L.

The undertaking of phytochemical studies of the herb, *Tragopogon orientalis* L. seemed worthwhile because of its cytochemical properties discovered recently, as well as its value as fodder. The chemical characteristic of this species, especially of its flavones, may also be of chemotaxonomic value.

MATERIAL AND METHODS

The material used in this study consisted of the leafy stems of *Tragopogon orientalis* L., collected from natural stands (a meadow and railway embarkment) in Motycz near Lublin, during blooming in June of 1979 and 1981. The stems were then dried under natural conditions and pulverized according to norms accepted for herbs (FP IV)).

The separation and purity of the compounds were controlled through thin-layer chromatography (TLC) on glass plates covered with a 0.25 cm layer of silica gel G Merck, or silica gel G with a 6% silver nitrate content. The chromatograms were developed using as the solvet phase (Krzaczek 1977, Wiłkomirski and Kasprzyk 1979):

F_1 : n-heptane-benzene-methanol, 50:50:0.5;
F_2 : petroleum ether-chloroform-acetic acid, 75:25:0.5;
F_3 : hexane-chloroform-methanol, 20:20:1;

and sprayed with the reagents (Borkowski 1973, Krzaczek 1977):

R_1 : concentrated H_2SO_4-ethanol, 1:1;
R_2 : anisaldehyde-acetic acid-methanol-concentrated H_2SO_4, 0.5:1:85:5;
R_3 : antimony pentachloride-chloroform, 25:75.

The spots on the chromatograms became visible after heating for 5 min. at 105°C.

Neutral aluminum oxide (POCH Gliwice) or silica gel G 35-70 mesh ASTM (Merck) impregnated with $AgNO_3$ (Wiłkomirski and Kasprzyk 1979) were used in column chromatography.

The unadjusted melting points were determined on a Boetius microscope table. ^1H NMR spectra were made using a BS 4870 NMR spectrophotometer and 50 mg of the studied compound dissolved in $CDCl_3$. Infrared spectrophotometric determinations were made in KBr using a Unicam SP 200 G spectrophotometer. Gas chromatrography was done on a Varian S2-8.

One kg of the raw material was extracted with petroleum ether (with a boiling point of 45-60°C) in a Soxhlet extractor for 60 h.

The solvent was evaporated from the extract. A greenish-brown residue was obtained, which was then dissolved in boiling ethyl ether. After cooling, a precipitate formed in the solution and was filtered off. Three grams of residue O and filtrate P were obtained.

Thin-layer chromatography of filtrate P revealed the presence of close to 20 compounds. On the basis of comparison (Rf values and spot color after developing with H_2SO_4) with the co-chromatographed standards, it was accepted that filtrate P contained free and estrified triterpenes and sterols. The compounds isolated from residue O and filtrate P were labeled A, B, C and D.

ISOLATION OF COMPOUND A

The primary chromatographic analysis of residue O (TLC: gel G, F_1, R_1) indicated that it was composed of slightly impure compound A. In order to purify it, residue O was applied to column I filled with neutral aluminum oxide. The column was successively eluted with: petroleum ether, pertoleum ether with benzene, 1:1, benzene, benzene with chloroform, 1:1. The composition of the eluates was checked chromatographically (TLC: gel G, F_1, R_1). Fractions 13-30 contained pure compound A, Rf 0.16 – the component of residue O.

ISOLATION OF COMPOUND B, TRITERPENE AND STEROL FRACTIONS

In order to obtain the fraction not subject to saponification, the ether was evaporated from filtrate P, and the dry residue (about 87 g) was subjected to alkaline hydrolysis (Jerzmanowska 1967). The hydrolysate was concentrated to about 1/3 of its volume, 0.5 dm^3 water was added and the components which did not undergo saponification were extracted with ether. The ether was evaporated. A semi-crystalline residue, 25.1 g, was obtained and dissolved in petroleum ether and applied to column II, filled with neutral aluminum oxide. The column was eluted successively using: petroleum ether, petroleum ether with benzene 5:1, 3:1, 2:1, benzene, benzene with chloroform 1:1, chloroform, chloroform with ethyl ether 1:1, ethyl ether. Sixty fractions were collected and their composition examined chromatographically (TLC: gel G, F_3, R_2). After the solvents were evaporated, the following were obtained: from fractions 1-5, pure compound B (5.4 g); from fractions 6-26 – a sediment of triterpenes T (12.2 g); from fractions 35-60 – a sediment of sterols S (5.9 g).

Sediment T gave in the Libermann-Burchard and Noller reactions colors characteristic for triterpenes (Jerzmanowska 1967).

Sediment S gave in the Libermann-Burchard and Salkowski reactions colors typical for sterols.

SEPARATION OF TRITERPENE FRACTION AND ISOLATION OF HOMOGENOUS COMPOUNDS C AND D

Sediment T was dried and acetylated (Jerzmanowska 1967). A small amount of the acetates was dissolved in chloroform and used for thin-layer chromatography (Table 1), which revealed the presence of five triterpene

acetates. The acetates were separated on column III filled with gel G impregnated with $AgNO_3$. The column was eluted with n-heptane-benzene-ethanol, 50:50:0.5. Twenty-one fractions were obtained. Chromatography conducted on plates covered with gel $G+AgNo_3$ (F_2, R_2) showed that eluates 5-12 contained a mixture of compounds with Rf values of: 0.24, 0.29, 0.34, 0.40 and 0.50. This mixture, after free evaporation of solvents, crystallized in the form of needles, prisms and posts. Eluates 15-21 contained compound C in pure form (0.5 g) which crystallized in the form of long needles from ethanol. Eluates 5-12 were separated on column IV filled identically as column III. The column was eluted with petroleum ether with benzene, 95:5, 90:10, 85:15, 80:20, 75:25 and 50:50. Forty fractions were obtained. Chromatography (TLC: gel $G+AgNO_3$, F_2, R_2,) showed that fractions 11-14 contained pure compound D (Rf = 0.47). Fractions 15-36 were a mixture of substances, the separation of which was not attained.

Table 1

Results of thin layer chromatography of the components of the triterpene T fraction after acetylation

Triterpene acetate	Rf		Color with		
	F_1	F_2	R_1	R_2	R_3
Triterpene D acetate	0.50	0.50	red	pink-violet	pink-violet
Triterpene X acetate	0.45	0.40	red-violet	pink-violet	pink-violet
Triterpene Y acetate	0.39	0.34	red-violet	violet	pink-violet
Triterpene Z acetate	0.32	0.28	violet	violet	pink-violet
Triterpene C acetate	0.23	0.24	yellow	yellow	violet
β-Amyrin acetate	0.50	0.50	red	pink-violet	pink-violet
Lupeol acetate	0.23	0.23	yellow	yellow	violet

F_1 – n-heptane-benzene-ethanol, 50:50:0.5; F_2 – petroleum ether-chloroform-acetic acid, 75:25:0.5;
R_1 – concentrated H_2SO_4-ethanol, 1:1; R_2 – anisaldehyde-acid-methanol-concentrated H_2SO_4, 0.5:1:85:5:
R_3 – $SbCl_5$-chloroform, 25:75.

THE ISOLATION OF STEROLS

The 5.9 g of sediment S which had been obtained were dissolved in hot 95% ethanol and an ethanol solution of digitonin was added in order to obtain sterol digitonates (Ludwiczak et al. 1965, Nerlo and Kosior 1977). Next, the sterol digitonates were decomposed with acetic anhydride (Jerzmanowska 1967). Sterol acetates (1.3 g) were obtained and then identified by gas chromatography. Chloroform solutions of sterol acetates and of standards were analysed. Used was 1.5% SE 30 on Chromabsorb G, a developing temperature of 100-299°C, temperature increase rate of 10° min^{-1} the N_2 flow at the input at a temperature of 200°C was 53 cm^3 min^{-1}, the paper shift rate was 50 cm h^{-1}.

THE IDENTIFICATION OF COMPOUNDS A, B, C, D AND STEROLS

Compound A

After crystallization from a mixture of chloroform and methanol, the crystals melted at a temperature of 79.5-80°C, which is in agreement with literature data for hexacosanol 79.5,-79.8°C (Karrer 1958), and 80°C (Dominquez and Hinojosa 1976).
Analysis of elements for the formula $C_{26}H_{54}O$, m. wt. 382.58:
calculated: 81.60% C, 14.22% H;
obtained: 81.77% C, 14.79% H.
The following spectra were obtained for compound A:
IR (KBr) – 3400 (OH), 2850, 1450, 1240, 1050, 720 cm^{-1},
^1H NMR ($CDCl_3$) – 3.6 (2H, t), 1.25 (48H, s), 0.85 (3H, t).
The IR and ^1H NMR spectra are in agreement with those from literature (Dominquez and Hinojosa 1979) for hexacosanol. Compound A was determined to be hexacosanol – "ceryl alcohol".

Compound B

Compound B crystallized from methanol in the form of shining flakes with a melting point of 65.5-66.5°C, which is in exact agreement with data from literature for n-triacontan 65.5-66.5°C (Karrer 1958) and 65.5-67.5°C (Dominquez and Hinojosa 1976).
Analysis of elements for the formula $C_{30}H_{62}$, m. wt. 422.80;
calculated: 85.22% C, 14.78% H;
obtained: 85.75% C, 14.52% H.
In order to confirm the structure, the following spectra were made for compound B:
IR (KBr) – 2900 ($-CH_3$), 1450, 1240, 1050, 720 ($-CH_2-$) cm^{-1},
^1H NMR ($CDCl_3$) – 1.25 (56H, s), 0.8 (6H, t).
The spectral analysis and other data indicated that compound B was n-triacontan.

Compound C

In the Libermann-Burchard reaction, compound C gave a red color. It crystallized from anhydrous ethanol in the form of needles with a melting point of 217-218.5°C. When mixed with standard lupeol acetate, it did not depress the melting point.
IR (KBr) – 2920, 1720 (OAc), 1465, 1390, 1280, 1040, 980 cm^{-1}.
Compound C was subjected to hydrolysis in a 10% alkoholic solution of KOH (Jerzmanowska 1967), which resulted in obtaining a free alkohol with a metling point of 209-210°C. The melting point of lupeol given in literature is

210-212°C (Onyanov et al. 1967) and 208-210°C (Wrzeciono 1965). The IR absorption spectrum of the obtained alkohol was identical with that of standard lupeol.
IR (KBr) – 3550 (OH), 2900, 1470, 1390, 1260, 1040, 980 cm^{-1}.
Compound C was determined to be lupeol acetate.

Compound D

In the Libermann-Burchard reaction, compound D gave a reddish to violet color. It crystallized from methanol in the form of long, colorless posts, which melted at a temperature of 238-240°C. When mixed with β-amyrin acetate obtained from *Viscum album* (Krzaczek 1977), it did not depress the melting point. The IR spectrum for compound D was identical to that of standard β-amyrin acetate.
IR (KBr) – 2950, 1720 (OAc), 1475, 1380, 1270, 1030, 990 cm^{-1}.
As the result of hydrolysis in 10% alcoholic KOH, a free alcohol was obtained,

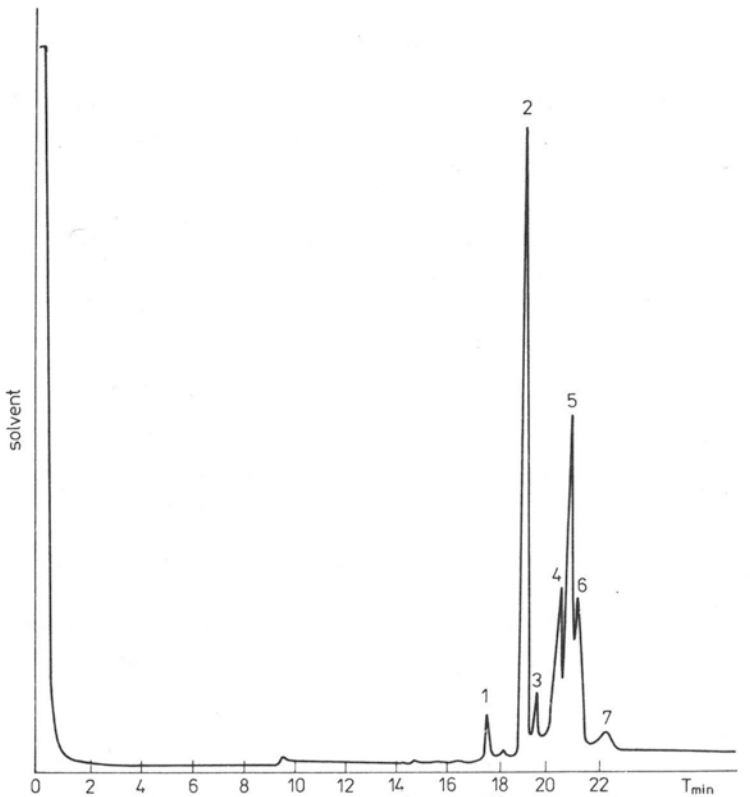

Fig. 1. Gas chromatography of sterol acetates. 3 – cholesterol acetate, 4 – stigmasterol acetate, 5 – β-sitosterol acetate, 1, 2, 6, 7 – unidentified sterol acetates

which after crystallization in methanol, melted at a temperature of 197-198°C. The infrared spectrum of the alcohol was identical with that of standard β-amyrin. IR (KBr) – 3550 (OH), 2950, 1475, 1390, 1250, 1030, 990 cm^{-1}.
Compound D was taken to be β-amyrin acetate.

Gas chromatography revealed the presence of 7 sterol acetates (Fig. 1), among which the following were identified by comparison of their retention times, R_T, with standards:
cholesterol acetate – R_T 19′ 45″,
stigmasterol acetate – R_T 20′ 39″,
β-sitosterol acetate – R_T 21′ 03″.

RESULTS AND DISUSSION

Compounds A, B, C and D were isolated in crystalline form from the herb, *Tragopogon orientalis* L. Compounds A and B were identified as hexacosanol and n-triacontan on the basis of agreement of melting points, analysis of their elementary composition, analysis of infrared and ^1H NMR spectra.
Compounds C and D were isolated from the triterpene fraction and identified as lupeol and β-amyrin. The obtained compounds exhibited identical melting points, IR spectra, and Rf values as standard lupeol and β-amyrin. We were not successful in obtaining terpenes X, Y and Z in homogeneous form.

The sterols, obtained by the digitonin method, were identified by gas chromatography. The presence of 7 sterol acetates was demonstrated, among which β-sitosterol, stigmasterol and cholesterol were identified.

n-Triacontan, β-amyrin, lupeol, β-sitosterol and cholesterol were found for the first time in a representative of the *Tragopogon* genus. Hexacosanol has already been isolated from *Tragopogon pratensis* (Zellner 1928).

REFERENCES

Borkowski B., 1973. Chromatorafia cienkowarstwowa w analizie farmaceutycznej. PZWL, Warszawa.

Dominquez X. A., Hinojosa M., 1976. Isolation of 5-hydroxy-7, 3', 4'-trimetoxy-flavone from *Turnera diffusa*. Planta Med. 30: 68-71.

Grynia M., 1974. Trujące i szkodliwe rośliny łąk i pastwisk. PWRiL, Poznań.

Jerzmanowska Z., 1967. Substancje roślinne. Metody wyodrębniania. PWN, Warszawa.

Karrer W., 1958. Konstitution und Vorkommen der organischen Pflanzen stoffe. Birkhäuser Verlag, Basel und Stuttgart.

Kucerov E. V., Siraeva S. M., 1980. Nektaroproduktivnost i sostav sakharov nektara nekotorykh dikorastushchikh medonosov Bashkirii. Rast. Res. 16: 523-530.

Kucowa I., 1972. W.: Flora Polska XIII. PWN, Warszawa-Kraków.

Kuzmanov B., Edreva A., Kozhuharov S., Simeonov S., 1970. Taxonomic studies on the Bulgarian species of *Tragopogon* L. III. A comparative chromatographic study of nine species. Fragm. Flor. Geobot. 16: 453-474.

Krzaczek T., 1977. Badania farmakobotaniczne podgatunków *Viscum album* L. III. Terpeny i sterole. Ann. Univ. M. Curie-Skłodowska. Lublin. Sectio D 32: 125-134.
Krzaczek T., Miłkowska J., Grzycka K., Sokołowska-Woźniak A., 1981. Badanie aktywności cytostatycznej frakcji octanowej i etanolowej. Ann. Univ. M. Curie-Skłodowska. Lublin. Sectio D 36: 125-132.
Ludwiczak R. S., Trzebny W., Życzyńska J., 1965. Składniki chemiczne wilczomlecza tyrlicza (*Euphorbia lathyris* L.) I. Niektóre składniki obojętne. Roczn. Chem. 39: 1233.
Moldenhawer K., 1953. Nowe rośliny oleiste. Przem. Spoż. 7: 58-62.
Mowszowicz J., 1967. Rośliny trujące. Wyd. Szk. Pedagog. Warszawa.
Nerlo H., Kosior A., 1977. Identyfikacja steroli mchu drabika drzewkowatego – *Climacium dendroides* L. Acta Polon. Pharm. 34: 89-92.
Onyanov I., Funtara D., Hinkov H., Panov P., 1967. Phytochemische untersuchungen an unteridischen teilen von *Trachomitum venetum* (L). Woodson. Planta Med. 15: 287-292.
Rutkowska B., 1984. Atlas roślin łąkowych i pastwiskowych. PWRiL, Warszawa.
Schlechtendal D. F. L., Langethal L. E., Schenk E., 1887. Flora von Deutschland. XXX. Gera – Untermhaus, Verlag von Fr. E. Köhler.
Wiłkomirski B., Kasprzyk Z., 1979. Free and ester-bound triterpene alcohols and sterols in cellular subfractions of *Calendula officinalis* flowers. Phytochemistry 18: 253-255.
Włodarczyk S., 1980. Botanika łąkarska. PWRiL, Warszawa.
Wrzeciono U., 1965. Trójterpeny i sterole roślinne. VI. Trójterpeny pięciocykliczne oraz β-sitosterol z liści dębu bezszypułkowego *Quercus sessilis* Ehrh. Roczn. Chem. 39: 385-390.
Zellner J., 1928. Ref. Beilsteius Handbuch der organischen Chemie 4 Auflage 2 Ergänzungswerk die Literatur von 1920-1929 Umfasend, herausgeben von der Deutsch. Chem. Gesell. bearbeitet von Richter F. 1: 471. Verlag J. Springer, Berlin, 1941.

Badania fitochemiczne ziela Tragopogon orientalis L. (Asteraceae). 1. Składniki ekstraktu eteru naftowego

Streszczenie

Z wyciągu eteru naftowego sporządzonego z ziela *Tragopogon orientalis* L. wydzielono frakcję nie ulegającą zmydleniu. Z frakcji tej wyodrębniono w stanie krystalicznym n-triakontan, heksakosanol, β-amyrynę i lupeol. Identyfikację przeprowadzono na podstawie wyników analiz elementarnych, temperatur topnienia, analiz spektralnych w IR i ^{1}H NMR, oraz porównawczej chromatografii (TLC) z odpowiednimi wzorcami. Wydzielone metodą digitoninową sterole oznaczono za pomocą chromatografii gazowej. Wykazano obecność 7 octanów steroli, z których zidentyfikowano β-sitosterol, stigmasterol i cholesterol. n-Triakontan, β-amyrynę, lupeol, β-sitosterol, stigmasterol i cholesterol stwierdzono w rodzaju *Tragopogon* po raz pierwszy. Heksakosanol był izolowany z *Tragopogon pratensis* (Zellner 1928).