ISOLATION AND IDENTIFICATION OF ANISALDEHYDE AND THREE ALKALOIDS FROM LEAVES OF *THALICTRUM MINUS* VAR. *MICROPHYLLUM*

KEMAL H. C. BAŞER

Faculty of Pharmacy, University of Anatolia, Tepebaşi, Eskişehir, Turkey

ABSTRACT.—Isolation from the leaves of *Thalictrum minus* L. var. *microphyllum* Boiss. of anisaldehyde (1) and the alkaloids thalactamine (2), takatonine (3), and the new base thalimicrinone (4) is reported.

In a previous publication, the isolation of thirteen alkaloids from the roots and rhizomes of *Thalictrum minus* L. var. *microphyllum* Boiss. was reported (1). Here, an investigation of the alkaloids from the leaves of the plant is described.

RESULTS AND DISCUSSION

From the chloroform-soluble tertiary alkaloid fraction extracted from the leaves were isolated *p*-anisaldehyde and three alkaloids. Silica gel column chromatography of the extract yielded, first of all, *p*-anisaldehyde (1), identified by comparison of its uv, ir, nmr, and mass spectral properties with an authentic sample. Its occurrence in *Thalictrum* has not been reported before.

Thalactamine (2) was also obtained and again identified from its spectral properties and tlc comparison with an authentic sample. The alkaloid has previously been found to occur in a variety of *T. minus* L. growing in Bulgaria (2, 4).

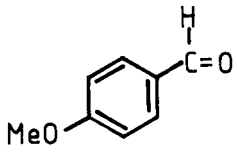
Takatonine (3) was the most abundant alkaloid in the leaves and although a quaternary base it comprised about two-thirds of the alkaloid fraction examined. It had earlier been isolated from the Japanese species *T. thunbergii* DC. (3, 5, 6).

The third alkaloid obtained, a minor component given the name thalimicrinone (4), proved to be a new compound. It is formulated as 1-(4'-methoxy-oxobenzyl)-5,6,7-trimethoxyisoquinoline, on the basis of the following evidence. The alkaloid showed absorption bands in the ir spectrum at ν (max) 1620 and 1660 cm^{-1} due to a conjugated double bond and a carbonyl group, respectively. In the nmr spectrum there was no signal corresponding to a *N*-methyl function. Four methoxy signals appeared at δ 3.89, 3.93, 4.03 and 4.08. In the aromatic region there were signals for seven protons in the form of one singlet and four doublets. One pair of doublets had a coupling constant of 6 Hz and the other pair of 9 Hz. *J* 6 Hz is characteristic of H-3 and H-4 of an isoquinoline ring system. The second pair of doublets, integrating to two protons each, belong to the A_2B_2 system of a *p*-substituted benzyl moiety. The mass spectrum indicated the molecular ion peak at *m/z* 353, which corresponds to the formula $C_{20}H_{19}NO_5$. The base peak appeared at *m/z* 135 and this fragment ion is also base peak in the *O*-methyl longifolonine (5) which has the same 4'-methoxy-oxobenzyl moiety (7).

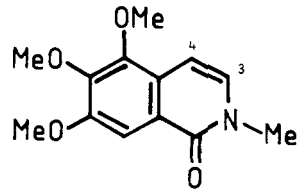
EXPERIMENTAL

INSTRUMENTS USED.—Uv spectra were taken on a Perkin-Elmer Lambda 3 recording spectrophotometer, and ir spectra were determined on a Unicam SP200 spectrometer. Nmr spectra were recorded on Perkin-Elmer R32 and Nicolet NT200 instruments. Mass spectra were measured with a VC Micromass 16F mass spectrometer. Melting points were determined in a Townson and Mercer melting point apparatus and are uncorrected.

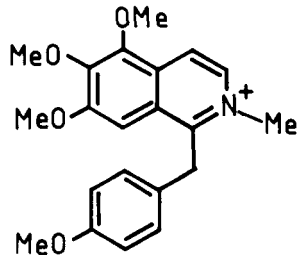
EXTRACTION.—Coarsely ground leaves (1.44 kg) of *Thalictrum minus* var. *microphyllum* collected in June 1979 from a meadow in Mahmudiye near Eskişehir, Turkey, were macerated with 1% and 2% acetic acid each time for 48 h. Precipitation of the alkaloids from the macerate with Mayer's reagent was followed by decomposition of the quaternary complex salts to chloride salts via passage of a solution of the precipitate in a mixture of acetone-methanol-water (6:2:1) through Amberlite IRA 400 (Cl^- form). The resultant aqueous solution was extracted first



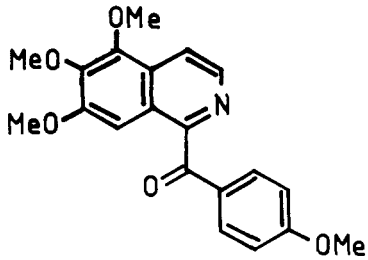
1



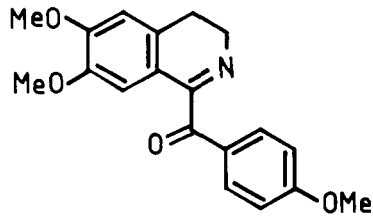
2



3



4



5

with light petroleum, which yielded a residue of 325 mg, then, after basification with 25% NH_4OH to pH 9, with ether (585 mg residue) and chloroform (9.37 g residue), alternately.

CHROMATOGRAPHY OF THE CHLOROFORM-SOLUBLE TERTIARY ALKALOID FRACTION.—The residue (2.95 g) was chromatographed over 200 g Si gel 60 (70–230 mesh ASTM) and eluted with chloroform (4 liters) and with chloroform containing the following amounts of methanol: 1% (2.2 liters), 2% (13.1 liters), 4% (5.7 liters), 8% (1.9 liters), 15% (1.3 liters), 30% (0.8 liter), 60% (0.6 liter) and methanol (1 liter). The column was finally stripped with 1% aq. HCl in methanol (0.4 liter). Fractions (100 ml) were collected and grouped after monitoring by tlc.

ANISALDEHYDE (1).—Fractions 1–5 eluted with chloroform yielded an oily principle with a pleasant smell. Preparative tlc of the oily residue on Si gel HF (0.75 mm) with chloroform as mobile phase resulted in the isolation of anisaldehyde as yellowish colored oil (47 mg); R_f 0.78 on Si gel HF with chloroform; uv (EtOH) 224, 240, 294, 304 (sh.), 318, 325 nm; ir (thin film) 1700 and 1690 (CHO), 850 (*p*-substituted benzene) cm^{-1}; nmr spectrum (90 MHz, $CDCl_3$) δ 3.88 (3H; *s*; OMe), 7.02 (2H; *d*, *J* 9 Hz; *ar*-H), 7.85 (2H; *d*, *J* 9 Hz; *ar*-H), 9.90 (1H; *s*; -C*H*O); ms *m/z* 137 (M^++1, 64%), 136 (M^+, C_8H_8O, 28), 135 (M^+−1, 100).

THALIMICRINONE (4).—The residue (20 mg) from fractions 6 and 7, also eluted with chloroform, was further separated by preparative tlc over Si gel HF (0.75 mm) with chloroform as mobile phase (run 2x). The only band which gave a positive reaction with Dragendorff's reagent was scraped off and eluted; it yielded 2 mg of colorless needles from abs. ethanol, mp 130–132°, R_f 0.35 on Si gel HF with chloroform; uv (EtOH) 210, 235, 250 (sh.), 295, 350 nm and a shift is observed upon addn. of dil. acid to 220, 245, 263 (sh.), 283, 315, 325 nm. No base shift is observed; ir (solid film) 1660 (C=O), 1620 (conj. double bond) cm^{-1}; nmr spectrum (250 MHz, $CDCl_3$) δ 3.89 (3H; *s*; OMe), 3.93 (3H; *s*; OMe), 4.03 (3H; *s*; OMe), 4.08 (3H; *s*; OMe), 6.96 and 7.96 ((2 x H; 2 x *d*, *J* 9 Hz; H–2', H–3' and H–5', H–6'), 7.37 (1H; *s*; H–8), 8.01 and 8.48 (2 x 2H; 2 x *d*, *J* 6 Hz; H–4 and H–3); ms *m/z* 353 (M^+, $C_{20}H_{19}NO_5$, 47%), 338 (45), 326 (38), 323 (23), 322 (39), 310 (27), 295 (14), 280 (11), 279 (8), 278 (13), 264 (11), 135 (100).

THALACTAMINE (2).—The residue (140 mg) from fractions 8–13, eluted with chloroform was purified by preparative tlc on Si gel PF (0.75 mm) with toluene-acetone-25% ammonium hydroxide solution (32:16:1) as mobile phase. The residue (90 mg) gave colorless plates from methanol, mp 112–114°; R_f 0.52 on Si gel HF with toluene-acetone-25% ammonium hydroxide solution (32:16:1); uv (EtOH) 240, 265, 278, 288, 312, 328, 340 nm, no change upon addn. of acid or base; ir (Nujol) 1660 (lactam carbonyl), 1620 (conj. double bond) cm^{-1}; nmr (90 MHz, $CDCl_3$) δ 3.60 (3H; *s*; NMe), 3.98 (9H; *s*; 3 x OMe), 6.72 and 7.01 (2 x H; 2 x *d*, *J* 7.5 Hz; H–4 and H–3)' 7.69 (1H; *s*; H–8); ms *m/z* 249 (M^+, $C_{13}H_{15}NO_4$, 30%), 83 (100).

TAKATONINE (3).—The residue (56 mg) from fraction 218, eluted with 4% methanol in chloroform, was purified by preparative tlc on Si gel HF (0.75 mm) with chloroform-methanol (9:1). A large band with a bluish white fluorescence under uv light was scraped off and eluted; it yielded a residue (18 mg) which gave yellow plates from methanol after treatment with 10% sodium iodide soln., mp (iodide) 181–182°; R_f 0.28 on Si gel HF with chloroform-methanol (8:2); uv (EtOH) 226, 265, 315, 365 nm, no change on addn of acid or base; ir (solid film) 1630, 1610, 1525, 1505, 1490, 1435, 1358, 1290, 1278, 1200, 1185, 1140, 1100, 1050, 1035, 965, 930, 840 cm^{-1}; nmr spectrum (90 MHz, $CDCl_3$) δ 3.76 (3H; *s*; OMe), 4.01 (3H; *s*; OMe), 4.08 (3H; *s*; OMe), 4.14 (3H; *s*; OMe), 4.68 (3H; *s*; N^+Me), 5.12 (2H; *s*; benzylic $-CH_2-$), 6.82 and 7.20 (2 x 2H; 2 x *d*, *J* 9 Hz; H–2', H–3' and H–5', H–6'), 7.44 (1H; *s*; H–8), 8.32 and 9.20 (2 x H; 2 x *d*, 9 Hz; H–4 and H–3); ms *m/z* 354 (M^+, $C_{21}H_{24}O_4N^+$, 21%), 353 (78), 339 (10), 338 (48), 323 (8), 307 (7), 294 (6), 292 (5), 280 (7), 249 (7), 232 (19), 209 (8), 176.5 (6), 122 (10), 121 (100). In addition, fractions 211–292, eluted with 2 to 30% methanol in chloroform yielded a residue (1.95 g) consisting mainly of 3.

Treatment of 3 with $NaBH_4$ in ethanol gave tetrahydrotakatonine as a colorless residue; R_f 0.82 on Si gel HF (0.75 mm) with chloroform-methanol (8:2); nmr spectrum (90 MHz, $CDCl_3$) δ 2.53 (3H; *s*; NMe), 3.57 (3H; *s*; OMe), 3.78 (3H; *s*; OMe), 3.84 (3H; *s*; OMe), 3.86 (3H; *s*; OMe), 5.88 (1H; *s*; H–8), 6.82 and 7.50 (2 x 2H; 2 x *d*, *J* 9 Hz; H–2', H–3' and H–5', H–6'); ms *m/z* 357 (M^+, $C_{21}H_{27}NO_4$, <1%), 236 (100).

ACKNOWLEDGMENTS

This investigation was supported by NATO Research Grant No. 029.81. The author thanks Dr. H. B. Dutschewska of the Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria, for a gift of thalactamine and Dr. N. G. Bisset and Dr. P. J. Hylands, both of the Department of Pharmacy, Chelsea College, University of London, U.K., for useful discussions.

Received 8 January 1982

LITERATURE CITED

1. K. H. C. Başer, *Doga Bilim Dergisi, Temel Bilm.*, **5**, 163–171 (1981).
2. N. M. Mollov and H. B. Dutschewska, *Tetrahedron Letters*, (24) 1951 (1969).
3. E. Fujita and T. Tomimatsu, *Yakugaku zasshi*, **79**(8) 1082–1086 (1959).
4. H. Dutschewska and N. M. Mollov, *Bulg Acad. Sci. Comm. Dept. Chem.*, **8**(1) 134–137 (1975).
5. S. Kubota, T. Masui, E. Fujita and S. M. Kupchan, *Tetrahedron Letters* (40) 3599–3602 (1965); *J. Org. Chem.*, **31**, 516–520 (1966).
6. T. Tomimatsu, S. Yamada and R. Yuasa, *Yakugaku zasshi*, **97** (2) 217–222 (1977).
7. I. R. C. Bick, T. Sevenet, W. Sinchai, B. W. Skelton and A. H. White, *Aust. J. Chem.*, **34**, 195–207 (1981).