

Isolation and identification of alkaloids from fruits of *Berberis thunbergii* DC.

B. BRÁZDOVIČOVÁ, D. KOŠTÁLOVÁ, J. TOMKO, and HWANG YANG JIN

*Department of Pharmacognosy and Botany, Faculty of Pharmacy,
Komenský University, 880 34 Bratislava*

Received 10 January 1979

Two major bisbenzylisoquinoline alkaloids, isotetrandrine and berbamine and three protoberberine type alkaloids berberine, palmatine, and jatrorrhizine were isolated from seeds of *Berberis thunbergii* DC. Berberine and oxyberberine, which is not present in the seeds, were isolated from the pericarp. Identity of the isolated substances was verified by spectral means (i.r., u.v., and mass spectrometry), optical rotation, and comparison with specimens.

Из семян растения *Berberis thunbergii* DC. выделены два главных алкалоида бисбензилизохинолиновой группы изотетрандрин и бербамин. Из околоплодников выделен основной алкалоид третичной фракции оксиберберин, который отсутствует в семенах этого растения и берберин. Из четвертичных оснований выделены из семян берберин, пальматин и ятторрицин. Основания идентифицированы сравнением с истинными образцами и на основании спектральных данных и оптического вращения.

Berberine, jatrorrhizine, and aporfine alkaloid magnoflorine were isolated from the underground and aerial parts of this plant together with tertiary bases oxyberberine, berbamine, oxyacanthine, and isotetrandrine [1, 2]. The presence of alkaloids in fruits has not been tested so far. From the chemotaxonomic point of view it is important to ascertain the presence of secondary metabolites in the individual parts of the plant and therefore we studied their occurrence in fruits. Alkaloids berberine, berbamine, palmatine, oxyacanthine, jatrorrhizine, magnoflorine, and columbamine are present in most species of the genus *Berberis* L. Six species, *B. vulgaris*, *B. tschonoskyana*, *B. laurina*, *B. laurica*, *B. amurensis*, *B. baluchistanica*, contain new isoquinoline alkaloids [3]. It has been found that roots and stems of *B. oblonga* and *B. integerrima* contain alkaloids in an approximately equal ratio, which is not the case with leaves [4, 5]. Oblongamine, a new type of monoquaternary oxyacanthine type [4] phenolic alkaloid and 2'-*N*-methylberbamine [5] were found in the portion of tertiary alkaloids from roots of *B. oblonga*; 2'-*N*-methylberbamine is the first monoquaternary phenolic bisbenzylisoquinoline base. Seeds and pericarps of *B. thunbergii* DC. growing in this country were

reported to contain palmatine and no magnoflorine in contrast to other parts of this plant.

Experimental

Melting points were determined on a Kofler block, i.r. spectra were measured with a Specord UV VIS (Zeiss, Jena) spectrometer, optical rotation with a Bendix, model 143 A, polarimeter, and mass spectra with an AEI-MS 902 apparatus. Solvent systems for thin-layer chromatography on silica gel (Lachema, Brno) and Silufol UV 254 (Kavalier, Votice) were: methanol—diethylamine 4 : 1 (S_1) and cyclohexane—chloroform—diethylamine 4 : 5 : 1 (S_2); detection of u.v. fluorescing alkaloids under u.v. light and with Dragendorf reagent.

Extraction and isolation of alkaloids

Fruits were collected in January 1977 in the city of Bratislava. Seeds were separated from the pericarp and each part was separately dried at room temperature. The ground seeds (2470 g) were stepwise extracted with light petroleum and methanol in a Soxhlet apparatus, the extract was concentrated under diminished pressure, dissolved in acetic acid (5%), filtered, made alkaline with NH_4OH to pH 8 and extracted with ether. The ethereal layer afforded after evaporation a mixture of tertiary alkaloids, the work-up of the aqueous layer gave a mixture of quaternary bases. Pericarp (2675 g) was worked up in the same manner.

The ethereal portion from seeds (1.64 g) was separated by column chromatography on basic alumina (Woelm, 150 g, activity grade I) in benzene, benzene—chloroform, chloroform—methanol, and methanol. Isotetrandrine (36 mg) was obtained from the chloroform (30%)—benzene eluent after crystallization from ether. M.p. 182°C , $[\alpha]_D^{23} = +160^\circ$ (c 0.21, CHCl_3), R_f 0.60 (S_2). Reported [6, 7] m.p. 182°C (benzene—light petroleum), $[\alpha]_D^{17} = +146^\circ$ (methanol) or $[\alpha]_D^{20} = +150.7^\circ$ (CHCl_3). The i.r. and u.v. spectra are identical with those given in the literature. Mass spectrum m/z 622.3042 (M^+) (for $\text{C}_{38}\text{H}_{42}\text{N}_2\text{O}_6$, calculated 622.3044), 485 ($M - 137$), 431 ($M - 191$) accords with the published data [8, 9].

Elution with methanol (10%)—chloroform furnished berbamine (59 mg) which showed a positive FeCl_3 reaction [10]. M.p. $128-130^\circ\text{C}$ (cyclohexane—benzene), $[\alpha]_D^{23} = +103^\circ$ (c 0.2, CHCl_3), R_f 0.25 (S_2). Reported m.p. $135-140^\circ\text{C}$ (benzene), $[\alpha]_D^{22} = +103^\circ$ (CHCl_3) [7]. The i.r. spectrum (KBr) displayed a band at 3450 cm^{-1} characteristic of a phenolic functional group. The u.v. spectrum (MeOH) showed maxima at 284 nm ($\log \epsilon$ 3.84), with addition of 0.01 M-NaOH at 287 nm ($\log \epsilon$ 4.10). Mass spectrum m/z 608.2875 (M^+) (for $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_6$, calculated 608.2886), 471 ($M - 137$) and 417 ($M - 191$) is identical with those of an authentic specimen and literature [9, 11].

To the aqueous layer after removal of the mixture of tertiary alkaloids, acidified with concentrated HCl to pH 4, a saturated solution of KI was added. Quaternary iodides of protoberberine alkaloids formed were extracted with chloroform, dried with Na_2SO_4 and evaporated to dryness (154 mg). Separation by column chromatography on neutral alumina (Reanal, 15 g, activity grade II) using chloroform, chloroform—methanol, and methanol as eluents afforded palmatine iodide from methanol (1%)—chloroform (9.1 mg). M.p.

225—228°C (water) and R_f value 0.16 (S_1) are in accordance with those of the specimen. Reported [12] m.p. 239—241°C (water). Maxima in the u.v. spectrum (228, 265, 350, 425 nm, $\log \epsilon$ 4.50, 4.48, 4.49, 3.82, respectively) are consistent with data reported [13]. The same eluent afforded berberine iodide (15.9 mg), m.p. 270—274°C (methanol), R_f 0.20 (S_1), m.p. and u.v. spectrum are identical with those of the specimen. Reported m.p. 262°C [12] (methanol). Elution with methanol furnished the third alkaloid of R_f 0.73 (S_1), (23 mg). Since this base failed to crystallize, it was reduced with Zn powder in acetic acid; the work-up afforded tetrahydrojatrorrhizine (2.2 mg). M.p. 205°C (methanol) and R_f 0.22 (S_2) agree with values of the specimen and literature [14].

Alkaloids in the pericarp

The content of the ethereal portion (1.25 g) was separated by column chromatography on neutral alumine (Reanal, 120 g, activity grade II) with the same solvents as before. From the fraction methanol (10%)—chloroform oxyberberine (6 mg) was obtained. M.p. 195—200°C (methanol), R_f 0.41 (S_2). Reported [11] m.p. 190—194°C (CHCl_3). The u.v. spectrum in methanol showed maxima at 228, 286, 315, and 345 nm ($\log \epsilon$ 4.58, 3.96, 4.32, 4.53) identical with those reported [11]. Mass spectrum m/z 351.1114 (M^+) (for $\text{C}_{20}\text{H}_{17}\text{NO}_5$, calculated 351.1107), 336 ($M - \text{CH}_3$), 322, 308, 292 agrees with the literature [11]. Further two alkaloids found in the pericarp are, basing upon their R_f values, identical with isotetrandrine and berbamine, isolated from the seeds. Oxyberberine is not present in seeds of this plant.

The aqueous layer after separation of tertiary alkaloids was made alkaline with 40% solution of NaOH (pH 10—12) and extracted with ether. Addition of crystalline citric acid to this ethereal solution resulted in separation of citrates of protoberberine bases, which were transformed into chlorides with dilute (1 : 1) hydrochloric acid. Crystallization from methanol gave berberine chloride (8.5 mg). M.p. 204°C and the R_f value are in accordance with the reported data [15].

Acknowledgements. Our thanks are due to Dr. L. Dolejš, DrSc., (Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague) for measurement and interpretation of mass spectra.

References

1. Tomita, M. and Kikuchi, J., *J. Pharm. Soc. Jap.* 76, 597 (1956).
2. Tomita, M. and Yang, T. H., *Yakugaku Zasshi* 80, 845 (1960).
3. Ikram, M., *Planta Med.* 28, 353 (1975).
4. Karimov, A., Telezhenetskaya, M. V., Lutfullin, K. L., and Yunusov, S. Yu., *Khim. Prir. Soedin.* 1, 80 (1977).
5. Karimov, A., Telezhenetskaya, M. V., Lutfullin, K. L., and Yunusov, S. Yu., *Khim. Prir. Soedin.* 2, 227 (1978).
6. Glasby, J. L., *Encyclopedia of the Alkaloids*. Plenum Press, New York, 1975.
7. Yuraphat, T., Pachaly, P., and Zymalkowski, F., *Planta Med.* 25, 315 (1974).
8. Shamma, M., *The Isoquinoline Alkaloids*. Academic Press, New York, 1972.

9. Tomita, M., Kikuchi, J., Fujitani, K., Kato, A., Furukawa, H., Aoyagi, Y., Kitano, M., and Ibuka, T., *Tetrahedron Lett.* 8, 857 (1966).
10. Chatterjee, R., Guha, M. P., and Das Gupta, A. A., *J. Indian Chem. Soc.* 29, 921 (1952).
11. Ikram, M. and Khan, F., *Planta Med.* 32, 212 (1977).
12. Cava, M. P. and Reed, T. A., *Lloydia* 28, 73 (1965).
13. Hruban, L., Šantavý, F., and Hegerová, S., *Collect. Czech. Chem. Commun.* 35, 3420 (1970).
14. Chatterjee, R., Guha, M. P., and Chatterjee, A., *J. Indian Chem. Soc.* 29, 371 (1952).
15. Wu-Nan, Wu and Beal, J. L., *Lloydia* 39, 204 (1976).

Translated by Z. Votický