

ALKALOIDS OF BERBERIS LYCIUM ROYLE-I

M. IKRAM, M. EHSANUL HUQ AND S.A. WARSİ

North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar

(Received August 3, 1965)

Three new alkaloids named as Berbenine, $C_{19}H_{21}NO_3$, m.p. 152-53°, Berbericine $C_{20}H_{17}NO_4$, m.p. 162-63° and Berbericinine iodide, $C_{21}H_{22}NO_4I$, m.p. 205-6° (dec.) have been isolated from *B. lycium*.

Introduction

Berberis lycium (Berberidaceae) grows wild round Murree hills. Concentrated semi-solid aqueous extract of the roots, called *Rasaut*, is a highly esteemed drug in the indigenous system of medicine as a bitter tonic and febrifuge. It is considered useful in the enlargement of liver and spleen and in the treatment of gastric and duodenal ulcers, as also in chronic diarrhoea and piles. Dried root powder taken with milk is considered very effective in the treatment of muscular pains and rheumatic conditions of the joints.¹ Chatterjee^{2,3} isolated umbellatine from the air-dried stem bark of *B. lycium* and *B. umbellata*. He reported a close resemblance of the ultra violet spectra of umbellatine with that of berberine isolated from European berberis. The salts of the two bases were also prepared and compared.

In view of the medicinal importance of the plant and the comparatively little work done, *B. lycium* roots were taken for detailed chemical examination. Moreover, the berberis are not easily distinguished from one another. Considerable ambiguity still exists particularly as most of them are known by the same vernacular name and are alleged to possess identical medicinal properties. The present work also indicated that the plant was not correctly identified by the Indian worker.

As a result of the present investigation three new crystalline alkaloids were isolated:

1. A white crystalline base provisionally named berbenine, $C_{19}H_{21}NO_3$; m.p., 152-53°C,
2. A yellow crystalline base, named berbericine, $C_{20}H_{17}NO_4$; m.p., 162-63°C (dec.).
3. A yellow crystalline base hydroiodide, named berbericinine hydroiodide, $C_{21}H_{22}NO_4I$; m.p., 205-6° (dec.).

Berbenine could be easily separated by basifying the crude aqueous extract with aqueous ammonia (10%) and extracting with ethyl acetate, Berbericine and berbericinine were precipitated as sparingly

soluble hydrochloride and hydroiodide respectively on saturating the mother liquor first with concentrated hydrochloric acid and sodium chloride and subsequently with potassium iodide.

Berbenine is optically active and melts at 152-53°. It has no methylene-dioxy group, the nitrogen group being tertiary in character. The presence of two methoxy and one N-Methyl group has been shown from N.M.R. UV spectra show that it has an aporphine⁴ structure. It has been formulated as $C_{19}H_{21}NO_3$ on the basis of combustion analysis and molecular weight determination. Two oxygen atoms are accounted for by two methoxy groups and the third possibly as -OH or -OC₂H₅. Infra-red spectra indicate the presence of -OH group (3320cm^{-1}) but it failed to form the acetyl derivative. Its hydrochloride, hydroiodide and methiodide could not be crystallised.

Berbericine hydrochloride on recrystallisation from ethyl alcohol gave yellow needles, m.p. 199-200°C (dec.) while the free base was recrystallised from ethyl acetate and melted at 163-64° (dec.) It is optically inactive, the nitrogen being tertiary in character. It contains two -OCH₃ and one N-methyl groups and has been assigned the formula $C_{20}H_{17}NO_4$. Two oxygen atoms are accounted for by two methoxy groups and the remaining two may be due to two hydroxy groups. The IR spectra also shows the presence of hydroxyl group (3400-cm^{-1}) The UV spectra show that it has a protoberberine⁵ structure. The IR spectra, molecular formula as well as the colour reactions of berbericine sulphate differ from the Infra-red spectra, molecular formula and the colour reactions of the authentic sample of berberine sulphate.

Berbericinine was isolated as hydroiodide which is formulated as $C_{21}H_{22}NO_4I$, on the basis of combustion analysis. It is optically inactive, nitrogen being tertiary in nature. It was found to contain three methoxy and one N-methyl groups. The fourth oxygen atom may be accounted for by one-OH group which is also indicated by its IR spectra (3400-cm^{-1}). It has

not been possible to prepare its free base or any other derivative.

The shade dried bark and the woody part of the stem of *B. lycium* did not show the presence of berbenine, but contained berbericine and berbericinine which could be separated as hydrochloride and hydroiodide respectively. The percentage of the bases which have been isolated separately from roots, barks and woods are noted below:

Plant	% of Berbenine	% of Berbericine hydrochloride	% of Berbericinine hydroiodide*
Roots	0.77	1.07	0.92
Barks	Nil	0.35	0.30
Woods	Nil	0.42	0.16

*Mixture of Berbericine and Berbericinine hydroiodide

Experimental

Analyses were carried out by Dr. Alfred Bernhardt, Mikroanalytisches Laboratorium, 433 Mulheim (Ruhr), West Germany and the Microanalytical Section, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi. IR and UV spectra were recorded by Mr. Iftikhar Ahmad, West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore and M. Eshaque, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi. Melting points were taken in Gallenkamp Micro melting point apparatus. All m.ps. are uncorrected.

The roots (9.03 kg.) were collected in the month of August-September from Nathiagali (West Pakistan) and were cut into 2-3 cm. bits and percolated with ethyl alcohol which was progressively diluted. In all, sixteen extracts were taken when the final strength of the alcohol used for extraction was 50%. It was found that berbenine was more soluble in concentrated alcohol while berbericine and berbericinine were easily extracted with dilute alcohol. The combined extracts were first concentrated in a cyclone evaporator and then under reduced pressure in order to remove the alcohol completely. The temperature was not allowed to rise above 70°. The aqueous residue thus obtained was basified with 10% ammonia and repeatedly extracted with ethyl acetate.

The ethyl acetate extract was dried over anhydrous sodium sulphate, treated with activated

charcoal and filtered. The solvent was removed completely under reduced pressure when light brown crystals of berbenine, m.p., 145-50°C were obtained (69.03 g.). On recrystallisation from ethyl alcohol, colourless shining needles of berbenine, m.p., 152-53°C were obtained.

Concentrated hydrochloric acid was then cautiously added to the well cooled mother liquor, followed by a saturated solution of sodium chloride and the sides of the flask were scratched and kept in the cold overnight when berbericine hydrochloride separated in yellow needles, m.p., 190-95°, (96.79 g.). This was recrystallised from ethyl alcohol m.p. 199-200° (dec.). Two more crops were obtained on addition of more concentrated hydrochloric acid and sodium chloride to the filtrate and keeping in the cold.

After filtering off berbericine hydrochloride, solid potassium iodide was added to the filtrate which on standing overnight in the cold deposited a mixture of berbericine and berbericinine hydroiodide (83.64 g.). Two more such crops were obtained on the addition of more solid potassium iodide in the filtrate and keeping for 2-3 days in the cold. The mixture was separated by repeated extraction with ethyl alcohol when berbericine hydroiodide being less soluble separated out first. This was recrystallised from dilute acetone (95%) when yellow needles of berbericine hydroiodide were obtained m.p. 269-70°C (dec.).

The ethyl alcohol filtrate on concentration and cooling gave orange needles of berbericinine hydroiodide which on recrystallisation from the same solvent finally melted at 205-6° (dec.).

Berbenine is a bitter alkaloid soluble in chloroform, ethyl acetate, benzene, ether, ethyl and methyl alcohol, sparingly soluble in petroleum ether and insoluble in hot or cold water. Infra-red spectra show the peaks at 770, 825, 840, 855, 878, 967, 1030, 1075, 1118, 1163, 1200, 1265, 1315, 1353, 1410, 1430, 1447, 1500, 1600, 2900, 3320 (OH) cm^{-1} (KBr pellet). Found: C, 72.01; H, 6.58; N, 4.76; N-CH₃, 10.22; OCH₃, 15.87. Mol. wt. (Rast), 301, (non-aqueous) 298.

C₁₉H₂₁NO₃ requires: C, 73.29; H, 6.80; N, 4.50; NCH₃, 9.32; 2OCH₃, 19.92; Mol. wt. 311.37.

$$[\alpha]_D^{27} + 98^\circ \text{ (C, 0.4292, methyl alcohol).}$$

$$[\alpha]_D^{27} + 113^\circ \text{ (C, 0.6432, chloroform).}$$

R_f, 0.91 (n-butanol, acetic acid, water; 4:1:5, Whatman paper No. I, temp. 30°C).

It has λ_{\max} 237, 283 $m\mu$ indicating an aporphine structure. N.M.R. spectra show singlet at 7.5 p.p.m. ($-NCH_3$ group) and singlet at 6.3 p.p.m. ($O-CH_3$, integral indicating two methoxy).

Berbericine was obtained by adding an excess of 10% aqueous sodium hydroxide to the solution of hydrochloride in acetone. On allowing the mixture to stand after scratching the sides fine rods of berbericine were obtained, m.p., 160-61° (dec.) which on recrystallisation from ethyl acetate gave light brown thin needles m.p. 162-163° (dec.). It is soluble in acetone, ethyl acetate, chloroform, benzene, ethyl and methyl alcohol, very slightly in ether and insoluble in petroleum ether. Found: C, 70.29; H, 5.80; N, 4.21; $20CH_3$, 15.55; NCH_3 , 11.42; Mol. wt. (non-aqueous) 334.

$C_{20}H_{17}O_4N$ requires: C, 71.63; H, 5.11; N, 4.18; $2OCH_3$, 18.50; NCH_3 , 8.65; mol. wt. 335.34.
R_f, 0.76 (n-butanol, acetic acid, water 4:1:5, Whatman paper No. 1, temp. 30°). O°, (C, 0.778, ethyl acetate).

Berbericine hydrochloride is soluble in acetone, methyl and ethyl alcohol, chloroform, boiling water and insoluble in ethyl acetate, benzene, ether and petroleum ether. The IR spectra show the peaks at 900, 910, 930, 975, 1035, 1100, 1143, 1240, 1280, 1340, 1370, 1400, 1520, 1575, 1600, 3235 (OH) cm^{-1} (KBr pellet). Found: C, 64.38; H, 5.04; N, 3.61; Cl, 9.47. $C_{20}H_{18}O_4NCl$ requires: C, 64.60; H, 4.84; N, 3.77; Cl, 9.55. R_f, 0.62 (n-butanol, acetic acid, water 10:1:5, Whatman paper No. 1, temp. 30°).

$$[\alpha]_D^{27} O^\circ, (C, 'O'.4888, ethyl)$$

Berbericine hydroiodide was prepared by dissolving the hydrochloride of the base in dilute acetic acid (10%) and adding an excess of potassium iodide. The crystalline deposit was filtered, washed thoroughly with water, dried and recrystallised from dilute acetone (95%), gave very fine yellow needles, m.p., 269-70° (dec). It is soluble in acetone, ethyl and methyl alcohol, boiling water and insoluble in ethyl acetate, chloroform, benzene, ether and petroleum ether. Found: C, 51.79; H, 3.70; N, 2.89; I, 27.60. $C_{20}H_{18}O_4NI$ requires: C, 51.83; H, 3.88; N, 3.02; I, 27.42. R_f, 0.79 (n-butanol, acetic acid, water, 4:1:5, temp. 30°, Whatman paper No. 1).

Berbericine picrate was prepared by combining alcoholic solution of the hydrochloride and picric

acid. The yellow powder obtained was crystallised from a mixture of absolute alcohol and acetone (70:30). The fine needles of berbericine picrate melted at 227-28°C (dec).

Berbericine nitrate and sulphate precipitated on the addition of cold conc. nitric acid and concentrated sulphuric acid to an aqueous solution of the base hydrochloride. The nitrate was crystallised from a mixture of absolute alcohol and acetone (70:30). The fine needles, m.p., 263-64°C (dec.) were obtained. Berbericine sulphate was washed thoroughly with water, acetone and absolute alcohol when the orange powder melted at 274-75°C (dec). The infra-red spectra of berbericine sulphate shows the peaks at 708, 830, 877, 905, 921, 960, 973, 1030, 1053, 1105, 1150, 1190, 1230, 1245, 1300, 1324, 1360, 1380, 1420, 1455, 1480, 1500, 1563, 1600, 1625, 2900, 3400 (OH) cm^{-1} (KBr pellet).

Nitroso berbericine was prepared by dissolving the berbericine hydrochloride in dilute acetic acid (10%) and gradually adding solid potassium nitrite to the ice cold solution. The resulting deep orange reaction mixture deposited long needles which were recrystallised from the mixture of ethyl alcohol and acetone (70:30) gave m.p. 239-40°C (dec).

Berbericine hydroiodide was repeatedly crystallised from ethyl alcohol until it finally melted at 205-6° (dec). It is slightly soluble in ethyl and methyl alcohol, acetone but more so in dilute alcohol (95%) and dilute acetone (95%), very slightly soluble in chloroform, ethyl acetate, boiling water and insoluble in benzene, ether and petroleum ether. The UV spectra shows maximum absorption at λ_{\max} 220, 265 and 345 $m\mu$ which indicated the protoberberine structure. The IR spectra shows the peaks at 813, 823, 865, 905, 970, 1018, 1070, 1110, 1140, 1180, 1220, 1240, 1270, 1335, 1363, 1450, 1503, 1560, 1600, 1625, 2950, 3400 (OH) cm^{-1} (KBr pellet). Found: C, 52.32; H, 4.55; N, 3.16; I, 29.01; NCH_3 , 10.07; OCH_3 , 21.53.

$C_{21}H_{22}O_4NI$ requires: C, 52.60; H, 4.59; N, 2.92; I, 26.51; NCH_3 , 6.05; $3OCH_3$, 19.41.

$$[\alpha]_D^{27} O^\circ, (C, 0.5256, ethyl acetate).$$

R_f, 0.84 (n-butanol, acetic acid, water; 4:1:5, Whatman paper No. 1, temp. 30°).

Molecular weight could not be taken neither by Rast Method nor by non-aqueous method as the alkaloid decomposed and is also insoluble in acetic acid.

THE COLOUR REACTIONS⁶ OF THE ALKALOIDS.

S. No.	Tests	Berberine	Berbericine	Berbericinine
1.	Concentrated HNO ₃ .	Light brown colour.	Light brown changes to orange.	Dark brown, lightens on standing changes to orange on addition of a few drops of concentrated H ₂ SO ₄ .
2.	Concentrated H ₂ SO ₄ .	No colour, develops; violet colour on the addition of a drop of concentrated HNO ₃ , changes to brown and finally yellow on standing sometimes.	Pale yellow turns deep brown and on addition of a drop of concentrated HNO ₃ changes to orange.	Yellow changes into brown after 6-7 min. and finally on standing for 10-15 min. becomes violet.
3.	Addition of solid K ₂ Cr ₂ O ₇ to the concentrated H ₂ SO ₄ solution.	Original pale yellow changes to redish brown after 5-7 min.	Dark brown.	Dark brown.
4.	Frohde's reagent.	Deep violet changes to dirty violet and finally light violet.	Light yellow changes to dark yellow.	Light green turns to dark green and finally violet.
5.	Mandelin's reagent.	Bluish green.	Bluish changes to dark brown.	Light green turns to dark brown finally dirty violet.
6.	A drop of chlorine or bromine water.	Pale yellow.	No change.	Original light yellow changing into orange after 10-15 min.
7.	FeCl ₃ +K ₃ Fe(CN) ₆	Prussian blue.	Prussian blue.	Prussian blue.
8.	Molybdic acid dissolved in conc. H ₂ SO ₄ .	Dirty violet changing into yellowish green.	Yellow, becomes yellowish green.	Bluish green changes to orange after 7-8 min.

Acknowledgement.—The authors wish to thank Mr. N.A. Malik and associates for collection of the plant material and Messers Nisar Ahmad and Gharib Ullah for help in the course of this work.

References

1. A.K. Nadkarni, *Dr. K.M. Nadkarni's Indian Materia Medica* (popular Book Depot., Bombay-7, Dhootopapeshwar Prakashan Ltd., Panvel 1954), vol. I, 3rd edn., p.p. 189-90.
2. R.N. Chopra, S.L. Nayar, I.C. Chopra, *Glossary of Indian Medicinal Plants* (Council of Scientific and Industrial Research, New Delhi, 1956), p. 36.
3. R. Chatterjee, *J. Indian Chem. Soc.*, **19**, 233-8 (1942).
4. Alfred W. Sangster and Kenneth L. Stuart, *Chem. Rev.*, **65**, 88 (1965).
5. Alfred W. Sangster and Kenneth L. Stuart, *Chem. Rev.*, **65**, 94 (1965).
6. K. Peach, M.V. Tracey, *Modern Methods of Plant Analysis*, (Springer-Verlag, Berlin, 1955), vol. IV, p. 387.