

readily determined. Since some sulfonamides, particularly sulfathiazole, in chloroform and chloroform-alcoholic solutions give a strong color with the cobalt-isopropylamine reagent, it should be reiterated that the assay must be carried out strictly as directed and not by attempting to dissolve the samples directly in chloroform.

2. It is not likely that toxic amounts of barbiturate would be taken by patients receiving energetic sulfonamide medication, nor is it likely that sulfonamides alone would be taken in sufficiently large doses to produce acute collapse. Nevertheless, the possibility must be faced that an occasional case will present itself to a hospital laboratory where diagnosis of acute sulfonamide poisoning might be complicated by a previous barbiturate medication. In such cases the urine and blood, when properly treated, may give a positive cobalt color test due to the presence of either sulfonamide or barbiturate. Even in these rare cases it should not be difficult to demonstrate the presence or absence of barbiturates.

In saturated chloroform-alcoholic solutions all three of the sulfonamides studied give positive reactions with the barium, lithium and isopropylamine tests (Koppanyi, *et al.* (2)), but positive barium and lithium tests are not obtainable if the sulfonamides are extracted from 2.0 per cent aqueous media with chloroform.

Since the maximum concentration of free sulfonamide to be expected in the urine or other tissue fluids under the most extreme conditions would not exceed 2.0 per cent, it follows that urines from patients receiving sulfonamides alone would be negative with the barium and lithium tests, and positive only with the isopropylamine test. Using the shake-out procedure, if the barium and lithium tests are positive, this indicates that barbiturates are present in the urine in addition to sulfonamides.

It is, therefore, easy to diagnose barbiturate poisoning and distinguish it from sulfonamide intoxication. The barium and lithium tests, which are not only qualitative but also approximately quantitative, should be performed first on body fluids of suspected cases, and only if these tests are positive should one proceed with the more quantitative isopropylamine tests.

Recently, all three tests have been performed on a number of pathological urines of treated and untreated patients. In no case was a positive test obtained unless the patient had received barbiturate medication. These findings will be reported in the near future.

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## Umbellatine from *Berberis insignis*, Hook. f.

By R. Chatterjee\*

It is well known that alkaloids occur in the species of *Berberis*, family *Berberidaceæ*. Table I is a résumé of the investigations recorded in literature.

The results show that several other alkaloids together with berberine occur in the species *Berberes*. Though several European varieties have been worked up, few references are found for Himalayan *Berberes*. Chopra and others (7) in their study of the medicinal plants which grow in the Himalayas mention the total alkaloid contents of *B. asiatica*, as a mixture of berberine and oxyacanthine. Because of their yellow stem, the *Berberes* are called in Sanscrit *Daruharidra* (yellow wood), an extract of which was used by the Hindus, Greeks and Arabs for medicinal purposes. The extract was commonly used in India as a remedy against malaria and diarrhea. It was also applied to any external inflammation and in the treatment of oriental sores (7).

Several varieties of Himalayan *Berberes* have been referred to by Chopra (13) as berberine-containing plants. *B. umbellata* Wall., growing in the Himalayas, at an altitude of 9000–11,000 ft. (14), showed the absence of berberine and yielded a new alkaloid, called umbellatine (12). It was suspected that other Himalayan *Berberes* also may not contain berberine at all. The present paper deals with the isolation and identification of umbellatine, in another species of Himalayan *Berberes*, *B. insignis*, Hook. f., which grows at an altitude of 7000–10,000 ft., in the humid forests of the Eastern Himalayas from Nepal to Bhotan (14).

I could procure only stem-barks for the extraction of the alkaloid. However, a gravimetric assay showed the presence of the largest amount of the alkaloid in the roots.

It was in the month of May that I obtained the stem-barks of *B. insignis*, Hook. f. grown at a place near Darjeeling, 6500 ft. above sea level. I could find only one

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Table I.—Alkaloids of *Berberis* Species

| Species                            | Alkaloid              | Empirical Formula   | Melting Point, ° C.     | References |
|------------------------------------|-----------------------|---|-------------------------|------------|
| <i>B. vulgaris</i> , L.            | Berberine             | C <sub>20</sub> H <sub>19</sub> O <sub>3</sub> N                  | 144                     | (1)        |
|                                    | Berberamine           | C <sub>18</sub> H <sub>19</sub> O <sub>3</sub> N                  | 156                     |            |
|                                    | Oxyacanthine          | C <sub>19</sub> H <sub>21</sub> O <sub>3</sub> N                  | 208-214 (Hesse)         |            |
|                                    | Columbamine           | C <sub>21</sub> H <sub>22</sub> O <sub>3</sub> NI<br>(the iodide) | 224 (the iodide)        |            |
|                                    | Palmatine             | C <sub>21</sub> H <sub>22</sub> O <sub>4</sub> NI<br>(the iodide) | 240 (the iodide)        |            |
|                                    | Jatrorrhizine         | C <sub>20</sub> H <sub>20</sub> O <sub>3</sub> NI<br>(the iodide) | 208-210 (the iodide)    |            |
|                                    | Unnamed base          | C <sub>19</sub> H <sub>22</sub> ON <sub>2</sub>                   | 256 (the hydrochloride) |            |
| <i>B. nervosa</i> , Pursh.         | Berberine             |   |                         | (2)        |
| <i>B. glauca</i> , D. C.           | Berberine             |   |                         | (3)        |
| <i>B. aquifolium</i>               | Berberine             |   |                         | (4)        |
|                                    | Berberamine           |   |                         |            |
|                                    | Oxyacanthine          |   |                         |            |
| <i>B. buxifolia</i> , Laun.        | Berberine             |   |                         | (5)        |
| <i>B. aetnensis</i>                | Berberine             |   |                         | (6)        |
| <i>B. asiatica</i>                 | Berberine             |   |                         | (7)        |
|                                    | Oxyacanthine          |   |                         |            |
| <i>B. thunbergii</i>               | Berberine             |   |                         |            |
| D. C. var <i>Maximowiczii</i>      | Oxyberberine          | C <sub>20</sub> H <sub>17</sub> O <sub>5</sub> N                  | 199-201                 | (8)        |
|                                    | Jatrorrhizine         |   |                         |            |
|                                    | Columbamine           |   |                         |            |
|                                    | Berberamine           |   |                         |            |
|                                    | Oxyacanthine          |   | 217 (Kondo and Tomita)  |            |
|                                    | Shobakunine           |   | 204-206 (the iodide)    |            |
|                                    | Tetrahydroshobakunine | C <sub>20</sub> H <sub>23</sub> O <sub>4</sub> N                  | 140                     |            |
| <i>B. Darwinii</i> , Hook.         | Berberine             |   |                         | (9)        |
| <i>B. heteropoda</i> , Schrenk.    | Berberine             |   |                         | (10)       |
|                                    | Berberamine           |   |                         |            |
|                                    | Oxyacanthine          |   |                         |            |
|                                    | Jatrorrhizine         |   |                         |            |
|                                    | Columbamine           |   |                         |            |
|                                    | Palmatine             |   |                         |            |
| <i>B. laurina</i> , Billb. (Thunb) | Berberine             |   |                         |            |
|                                    | Hydrastine            | C <sub>21</sub> H <sub>21</sub> O <sub>6</sub> N                  | 132                     | (11)       |
| <i>B. umbellata</i> , Wall.        | Umbellatine           | C <sub>21</sub> H <sub>21</sub> O <sub>8</sub> N                  | 206-207 (decomp.)       | (12)       |

alkaloid, whose analytical data and properties and derivatives showed it to be identical with the umbellatine found by me in *B. umbellata*, Wall (12).

Table II.—The Total Alkaloid in *B. insignis*, Hook. f.

|            |               |
|------------|---------------|
| Stem-wood  | 0.38 per cent |
| Stem-bark  | 1.52 per cent |
| Whole root | 2.50 per cent |

## EXPERIMENTAL

*Preliminary Test for Alkaloids.*—About 10 Gm. of powdered stem-barks were extracted with hot alcohol. The residue from the alcoholic extract was treated with water, filtered from resinous matter and then treated with dilute hydrochloric acid. The solution was tested with alkaloidal reagents, such as Mayer's reagent, picric acid and Wagner's reagent. The tests were all positive.

*Systematic Examinations.*—Fifty Gm. of powdered stem-barks were extracted in a glass Soxhlet successively with petroleum ether (b. p. 40-60° C.), ether, chloroform and absolute alcohol.

*Isolation of an Alkaloid and Its Purification.*—For isolation of an alkaloid a quantity of 1 Kg. of powdered

Table III.—Results of the Extraction by Different Solvents

| Extract         | Amount, Per Cent | Nature   |
|-----------------|------------------|--|
| Petroleum ether | 3.5              | A green sticky mass consisting mainly of chlorophyll and waxy matter |
| Ether           | 0.9              | Waxy matter  |
| Chloroform      | 3.3              | A brownish sticky mass giving very faint reactions for alkaloids     |
| Alcohol         | 27.6             | A brownish sticky mass giving very strong reactions for alkaloids    |

air-dried stem-bark was percolated exhaustively with 98% ethyl alcohol. The alcohol of the extract was removed and the residue treated with 500 cc. of water and filtered to get rid of resinous matter. The filtrate was treated with hydrochloric acid to give a 1% solution of the aqueous extract, and allowed to stand for 2 hours. The hydrochloride of the alkaloid gradually crystallized out and was filtered off.

The crude hydrochloride was purified by crystallizing twice from hot water and finally from hot alcohol. The yellow silky crystals of the pure hydrochloride were then dissolved in hot water and a

dilute solution of sodium carbonate added until alkaline. The yellow color of the solution changed to deep brown and on cooling yellow silky needles of the free alkaloid were obtained. They were purified by repeated crystallizations from hot water and finally from hot alcohol. (Yield, 10 Gm.)

The alkaloid melted at 205–207° C. (decomp.). Repeated crystallizations of the alkaloid did not change its melting point. The results of combustion analyses conform to the empirical formula,  $C_{21}H_{21}O_3N$ .

*Analysis*.—Calculated for  $C_{21}H_{21}O_3N$ ,  $\frac{1}{2}H_2O$ : C, 59.4; H, 5.2; N, 3.3. Found: C, 59.0; H, 5.3; N, 3.3.

Umbellatine mixed with the alkaloid did not depress the melting point of the latter. Mixed melting point of nitrosoumbellatine and nitroso derivative of the alkaloid remained unchanged at 265–267° C. (decomp.). The amount of platinum in the chloroplatinate was found to be 15.63%, whereas platinum present in umbellatine chloroplatinate was determined as 15.71% (12). Calculated for  $(C_{21}H_{21}O_3N, HCl)_2PtCl_4$ : Pt, 15.72%. The coincidence of these results led me to conclude that umbellatine is present in *B. insignis*, Hook. f.

*Umbellatine Sulfate*.—Umbellatine sulfate is prepared by the usual method. Samples obtained from both sources melt at 274° C. (decomp.).

*Umbellatine Picrate*.—On treating a solution of umbellatine in alcohol, with an alcoholic solution of picric acid, the picrate was precipitated at once. The picrate was crystallized from hot alcohol as brown feathery needles. It melts at 232° C. (decomp.).

*Analysis*.—Calculated for  $C_{27}H_{24}N_4O_{15}$ : N, 8.7; Found: N, 8.9.

#### SUMMARY

1. Berberine and associated alkaloids were found to be present in many species of *Berberis*. Himalayan *Berberis*, however, showed the absence of berberine and yielded a different alkaloid.

2. One of the Himalayan *Berberis*, *B. insignis*, Hook. f., contains 1.0% of total alkaloid.

3. An alkaloid has been isolated and is found to be identical with umbellatine,  $C_{21}H_{21}O_3N$ , the alkaloid of *B. umbellata*, Wall., a Himalayan *Berberis*.

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## The Stabilization of Liquid Preparations Containing Pepsin\*

By C. J. Klemme† and C. L. Boswell‡

#### INTRODUCTORY

The present work is a continuation of the study of liquid preparations containing pepsin for the purpose of stabilizing the activity of peptic systems. Heretofore, as mentioned by Klemme and Worrell (1), stability studies of pepsin preparations have been inconclusive, due primarily to the lack of a satisfactory method of assay. Klemme and Worrell had this objective in mind, namely, to develop a procedure which could be employed in the accurate assay of pepsin preparations for activity. In the experiments conducted in this research their method of assay has been used entirely.

In the past few years several enzymes have been isolated in pure crystalline form and their chemical nature studied. Pepsin, the enzyme with which we are primarily con-

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