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RHETSINE AND RHETSINININE

THE QUINAZOLINE ALKALOIDS OF *XANTHOXYLUM RHETSA**†

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Abstract—From the trunk bark of *Xanthoxylum rhetsa* DC., lupeol and four alkaloids, namely, chelerythrine, rhetine, rhetsine and rhetsinine have been isolated. The constitution of rhetsinine and rhetsine have been established; the former is a new indolequinazoline base, the latter being its desoxy derivative.

THE chemical investigation of *Xanthoxylum rhetsa* DC. (Syn. with *X. budrunga* Wall; Fam. *Rutaceae*) has been undertaken and from the trunk bark of this species four alkaloids *viz.*, rhetsine, $C_{19}H_{17}N_3O$ (yield 0·03%), m.p. 271° (dec), rhetsinine, $C_{19}H_{17}N_3O_2$ (0·05%), m.p. 192° (dec), rhetine (0·01%), m.p. 254° and chelerythrine, $C_{21}H_{19}NO_5$ (0·014%), m.p. 210°, have been isolated in addition to lupeol[1] (0·7%). Rhetsine and rhetsinine are indolequinazoline alkaloids which have been observed so far only in *Evodia rutaecarpa*.[2] The chemistry of rhetsine and rhetsinine is described in the present communication.

Rhetsinine has the molecular formula, $C_{19}H_{17}N_3O_2$ and is a monoacidic tertiary base forming a monomethiodide and only one series of salts with hydrochloric, nitric and picric acids. Rhetsinine yielded a crystalline chloroplatinate. Both the base and its hydrochloride were optically inactive. It contained one iminomethyl and at least one active hydrogen but lacked in methoxyl, methylenedioxy, sidemethyl and carbonyl groups (as indicated from its indifferent behaviour towards carbonyl reagents). Catalytic reduction of rhetsinine furnished a new colourless compound, deoxyrhetsinine, $C_{19}H_{17}N_3O$, m.p. 271° (dec) which was also obtained from the parent base during its reduction with sodiumborohydride. I.R. spectrum of deoxyrhetsinine lacked the peak absorption for hydroxyl at 2·9 μ but retained all other absorption bands discernible in the infra-red spectrum of the original base. These data eventually proved that rhetsinine is a carbinolamine compound. This is in consonance with the analyses of its salts, the latter being formed from the base with a loss of one molecule of water.

Alkaline hydrolysis (30 per cent alcoholic potassium hydroxide) degraded the alkaloid to N-methylanthranilic acid, $C_8H_9NO_2$ (I) and a base, $C_{11}H_{10}N_2O$. The base was dihydropyrid-[3,4-b]-indole-1(2)one[3] (II) was settled by the fact that upon dehydrogenation with palladium black it produced pyrid-[3,4-b]-indole-1(2)-one[4] (IIa). Upon fusion with alkali rhetsinine decomposed into indole-2-acid (IIb) and pyrid-[3,4-b]-indole-1(2)-one (IIa) besides several indolaceous fragments which are under

* Part I in *Xanthoxylum* series.
† This paper is dedicated to Professor P. Karrer on the occasion of his 70th Birthday Celebrations.

[1] A. Chatterjee and C. Ghosh, *Ind. Sci. Congress* 44th Proc. Part III, p. 124.
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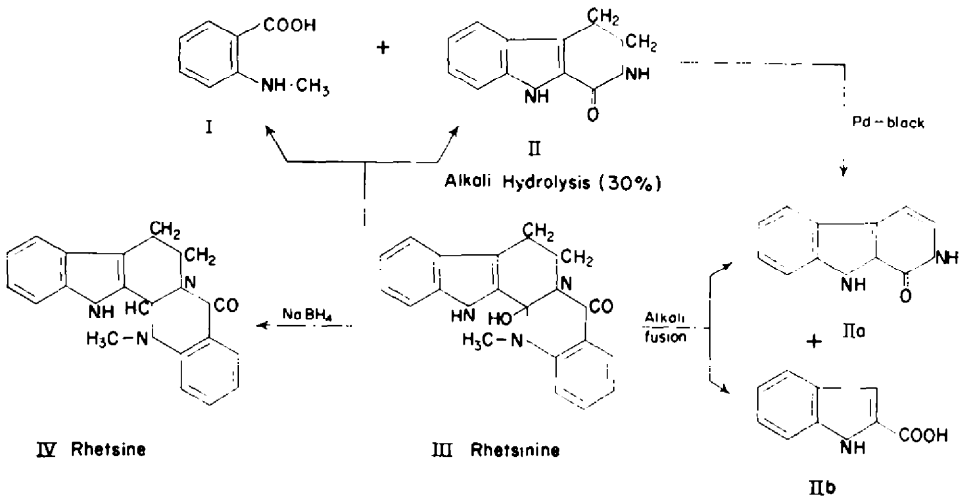
[3] R. H. F. Manske and R. Robinson, *J. Chem. Soc.* 240 (1927).
[4] E. Schlittler, H. U. Huber, F. E. Bader and H. Zahnd, *Helv. Chim. Acta* 37, 1912 (1954).



characterization. Isolation of N-methylanthranilic acid and dihydropyrid [3,4-b]-indole-1(2)-one from alkali hydrolysates of the base and indole-2-acid and pyrid [3,4-b]-indole-1(2)-one from alkali fusion products established the following indole-quinazoline structure III for rhetsinine and IV for deoxyrhetsinine.

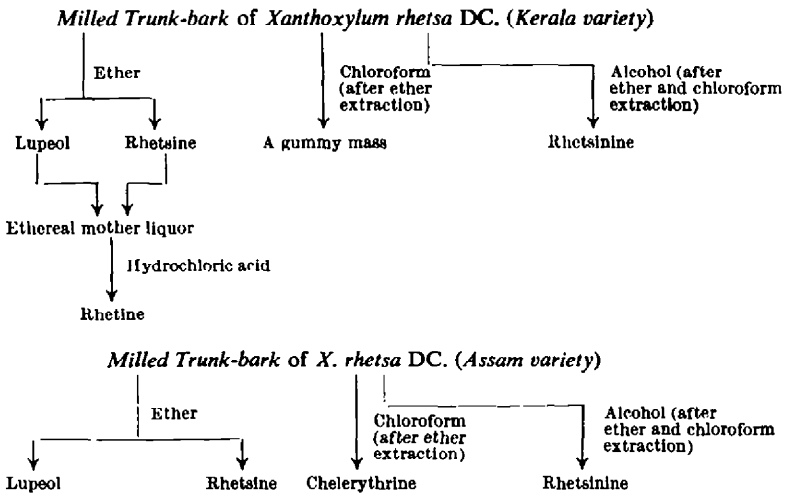
From the elementary analyses, colour reactions and properties (both physical and chemical) rhetsine, $C_{19}H_{17}N_3O$, m.p. 271° (dec) appeared to be identical with deoxyrhetsinine. This was subsequently confirmed from their mixture melting point determination and from a comparison of their U.V. and I.R. spectra. Hence the formulation of rhetsine stands as IV. Thus simultaneous occurrence of rhetsinine and its deoxy-derivative (= rhetsine) in the trunk-bark of *X. rhetsa* appeared to be of great interest from the view point of biogenesis. The constitution of the third alkaloid, rhetine, is in progress. The fourth alkaloid, $C_{21}H_{19}NO_5$ of *X. rhetsa* could be recognized as chelerythrine from its elementary analyses, from an examination of its functional groups and several derivatives as also from the analysis of its dihydro (= deoxy) derivative, $C_{21}H_{19}NO_4$ prepared from the base by reduction with sodium borohydride. The quaternary nature of this alkaloid was revealed from the analysis of its hydrochloride, $C_{21}H_{18}NO_4Cl$, m.p. 210°, and picrate, $C_{21}H_{17}NO_4C_6H_2OH(NO_2)_3$, m.p. 236° (dec), which formed with elimination of a molecule of water. The identity of this alkaloid with chelerythrine could finally be established by a direct comparison of the alkaloid concerned with an authentic sample of chelerythrine and their derivatives.

EXPERIMENTAL*

Isolation. Both Kerala (South India) and Assam (North India) varieties of *X. rhetsa* have been examined. Kerala variety produces rhetsine, rhetsinine and rhetine but no chelerythrine which occurs in Assam species but the latter lacks rhetine.

3 kg milled trunk-bark of *X. rhetsa* was extracted exhaustively with 3 l. ether in a soxhlet for 48 hr. During extraction lupeol and rhetsine (A) deposited in the extraction flask. A further crop of lupeol and rhetsine (B) was obtained from ether concentrate when kept for a fortnight in the frigidaire. Residual ether solution (250 cm^3) was diluted with another 300 cm^3 ether and shaken with 2 N HCl (50 cm^3 × 3) when rhetine, m.p. 238–242° was precipitated. After repeated crystallizations from methanol and ethanol, m.p. of rhetine could be raised to 254°.

* U.V. spectra have been studied in spectral alcohol with Beckman spectrophotometer Model DU. I.R. spectra have been examined in Nujol. For analysis all the samples have been dried in high vacuum at 100° for 12 hr.



The bark materials left after ether extraction were percolated with 3 l. hot chloroform and then refluxed with 3 l. alcohol for 64 hr. Acid extract of chloroform solution of Kerala species gave negative tests for alkaloids but that of Assam sample yielded chelerythrine chloride (C, 0·42 g).

Alcoholic extract of the bark was concentrated to 250 cm³ which was poured into 1 l. water acidulated with 1% acetic acid and left overnight. The clear orange decant was basified with sodium carbonate. The base liberated was taken up in chloroform (75 cm³ × 4). From the chloroform concentrate (50 cm³) rhetsinine separated out in reddish yellow rhombic plates, m.p. 186–190° (dec) which changed to yellow on keeping. After several crystallizations from the same solvent and from a mixture of chloroform and ethanol (1 : 3) pure rhetsinine, m.p. 192° (dec), $[\alpha]_D^{25°} = \pm 0°$ was obtained (1·5 g). It is sparingly soluble in ethanol, methanol, benzene, ethyl acetate, acetone, readily soluble in chloroform, and insoluble in water, dilute acid and alkalies. Thus alkaloid tests of the base could only be performed in conc HCl in which it is soluble. Paper chromatogram of the base was examined on a formamide impregnated paper using n-butanol : hydrochloric acid : water (100 : 1 : 30) ($R_f = 0{\cdot}55$ at 31°) and also n-butanol : formic acid : water (12 : 1 : 7) ($R_f = 0{\cdot}59$ at 31°) as developers. A single fluorescent spot appeared on the chromatogram. (Found: C, 71·47; H, 5·50; N, 13·05; H^+, 0·20; (N)—CH_3, 4·24; mol. wt. 314. (Rast). Calc. for $C_{19}H_{17}N_3O_2$: C, 71·47; H, 5·33; N, 13·16; H^+, 0·30; (N)—CH_3, 4·70%. mol. wt. 319.) The alkaloid was indifferent towards ferric ion and Tollens reagent, and does not reduce Fehling's solution. It turned deep yellow with conc H_2SO_4, greenish yellow with conc HNO_3, light yellow with Fröhde's reagent. U.V.: λ_{max} 314 mμ (log ε 4·15). I.R.: 2·9 (—OH), 3·03 (—NH), 5·9 (amide) and 6·03 μ (phenyl nucleus).

Rhetsinine chloride. 30 mg base in 10 cm³ chloroform was treated with 0·5 cm³ 2 N HCl and shaken vigorously, when hydrochloride of the base separated in yellow flocky mass. It crystallized from ethanol in yellow needles, m.p. 228–229°, (dec) $[\alpha]_D^{25°} = \pm 0°$. (Found: N, 12·44; Cl, 10·52. Calc. for $C_{19}H_{16}N_3OCl$: N, 12·51; Cl, 10·88%.)

Rhetsinine methiodide. 2 cm³ methyliodide was added to 30 mg base in 10 cm³ chloroform and kept overnight when long yellow slender needles of methiodide of the base separated. It was further purified by crystallization from ethanol, m.p. 220–221° (dec). (Found: N, 9·48. Calc. for $C_{20}H_{20}N_3O_2I$: N, 9·11%.)

Rhetsinine nitrate. 25 mg base hydrochloride in 20 cm³ ethanol was treated with 4 N HNO_3 till the precipitation was complete. The nitrate crystallized from ethanol in light-orange yellow needles, m.p. 242–243° (dec). (Found: N, 15·79. Calc. for $C_{19}H_{16}N_4O_4$: N, 15·39%.)

Rhetsinine picrate. 30 mg base hydrochloride was dissolved in 12 cm³ water in which 30 mg picric acid in 5 cm³ water was slowly added. The precipitated yellow picrate crystallized from ethanol in orange-yellow slender needles, m.p. 270–272° (dec). (Found: N, 15·27. Calc. for $C_{25}H_{20}N_6O_9$: N, 15·31%.)

Rhetsinine chloroplatinate. 80 mg base hydrochloride was dissolved in 50 cm³ hot water. The solution was cooled and treated with 5% chloroplatinate solution with stirring till the precipitation

was complete. The bright orange crystalline chloroplatinate was too sparingly soluble to be crystallized from any solvent. A pure sample when heated charred at 230° without melting. (Found: Pt, 19·05. Calc. for $(C_{19}H_{17}N_3O_2)_2$. H_2PtCl_6: Pt, 18·60%.)

Sodium borohydride reduction of rhetsinine. 1·0 g rhetsinine was dissolved in 150 cm³ chloroform–ethanol mixture (1 : 2). To this orange-yellow solution, 600 mg finely powdered sodium borohydride was added. There was copious evolution of hydrogen and the solution became colourless. Deoxyrhetsinine slowly separated after some time. It was left overnight at ordinary temp. Excess of the solvent was removed. The residue was treated with 50 cm³ water and extracted exhaustively with 500 cm³ chloroform. The chloroform extract after being distilled left a colourless solid (700 mg) which crystallized from chloroform–ethanol mixture in shining rectangular plates m.p. 270–271° (dec), $[\alpha]_D^{28°} = \pm 0°$. Deoxyrhetsinine turned red colouration with conc H_2SO_4, red to deep brown with conc H_2SO_4 and potassium dichromate, orange-red with Fröhde's reagent, red with conc H_2SO_4 and ceric sulphate, conc H_2SO_4 and *p*-dimethylaminobenzaldehyde, conc HNO_3, conc H_2SO_4 and ammonium molybdate and pink to violet with vanillin and conc HCl. (Found: C, 74·61; H, 6·00; N, 13·88; (N)—CH_3, 5.00; H^+, 0·30. Calc. for $C_{19}H_{17}N_3O$: C, 74·75; H, 6·22; N, 13·77; (N)—CH_3, 4·91; H^+, 0·33%.) U.V.: λ_{max} 266 mμ (log ε 4·08), 312 mμ (log ε 4·08) and 324 mμ (log ε 3·29). Deoxyrhetsinine is found to be identical with rhetsine, m.p. 271° (dec), another alkaloidal constituent of *Xanthoxylum rhetsa* DC., from an examination of their mixed m.p., U.V. and I.R. spectra.

Catalytic hydrogenation of rhetsinine. 220 mg rhetsinine dissolved in 25 cm³ glacial acetic acid was hydrogenated in presence of 70 mg Adam's platinum oxide catalyst for 9 hr at 28°. The uptake of hydrogen was slow and at the end it consumed only 20 cm³ hydrogen. The acetic acid was removed under reduced pressure and the concentrate was cooled to 0° and poured in 25 cm³ water. The resulting turbid solution was basified when a light yellow flocculent precipitate separated which was taken up in 50 cm³ chloroform. The latter upon concentration and dilution with ethanol produced yellowish brown solid melting at 245–253° (dec). From chloroform–ethanol mixture (1 : 2) it separated in rectangular colourless plates, m.p. 265–267° (80 mg). Further crystallization from methanol–acetone mixture raised the m.p. to 271° (dec). It was found to be identical with deoxyrhetsinine prepared from the base by reduction with sodium borohydride.

Alkali hydrolysis of rhetsinine. 2·0 g rhetsinine was boiled under reflux with 75 cm³ 30% alcoholic caustic potash for 5 hr. The pale yellow hydrolysate was distilled under reduced pressure to remove ethanol, when a yellow oil separated. This was diluted with 25 cm³ water and extracted with 300 cm³ ether. The aqueous portion (P) was kept. The ethereal extract was washed with water, dried and concentrated when dihydropyrid-[3,4-b]-indole-1(2)-one was obtained (30 mg). The compound melted at 172° and gave a blue-violet colour with Fröhde's reagent and a dark-brown colour with $K_2Cr_2O_7$ and conc H_2SO_4, reminiscent of a β-carboline compound. (Found: C, 71·10; H, 5·49; N, 15·20. Calc. for $C_{11}H_{10}N_2O$: C, 70·96; H, 5·37; N, 15·05%.)

Conversion of dihydropyrid-[3,4-b]*-indole-*1(2)*-one into pyrid-*[3,4-b]*-indole-*1(2)*-one.* 20 mg substance was intimately mixed up with 100 mg palladium black and the mixture was heated in a distillation bulb at 190–195° for 45 min. It was then sublimed *in vacuo* at 160°/0·01 mm when a solid mass was obtained. This was twice crystallized from benzene when silky colourless needles, m.p. 259°, separated. (Found: C, 71·59; H, 4·21; N, 14·98. Calc. for $C_{11}H_8N_2O$: C, 71·73; H, 4·34; N, 15·21%.)

Isolation of N-*methylanthranilic acid.* The aqueous alkaline solution (P; *vide supra*) left after ether extraction of alkali hydrolysates was cooled and carefully acidified with dil acetic acid. A yellowish-white precipitate appeared at pH 6 which was taken up in 300 cm³ ether. The ethereal layer which exhibited a strong bluish-violet fluorescence was washed, dried and concentrated when a yellowish product was obtained. It crystallized from petroleum ether (B.P. 40–60°) and absolute ethanol mixture (2 : 1) in colourless needles, m.p. 179–180° (750 mg). (Found: C, 63·45; H, 6·01; N, 9·36. Calcd. for $C_8H_9NO_2$: C, 63·57; H, 5·96; N, 9·27%.) This did not depress the melting point of an authentic sample of N-methylanthranilic acid on admixture.

Alkali fusion of rhetsinine. 500 mg rhetsinine was fused with 3·5 g caustic potash in a nickel crucible heated to 300–310° for 15 min. The fused mass was well stirred to prevent the frothing up of the molten mass due to the copious evolution of gases possessing an indolaceous odour. It was cooled, lixivated with 20 cm³ water, 2·0 g solid NH_4Cl was added and the resulting solution was extracted exhaustively with ether. The ether extract containing basic and neutral fractions was

freed from the solvent and the residue on sublimation *in vacuo* at 0·01 mm gave two different fractions (X) at 66–80° and (Y) at 150–160°. Fraction (X) had a faecal smell and consisted of a few drops of reddish-yellow oil. It gave a positive pine chip test for indole. Fraction (Y) solidified on keeping. This on being crystallized from benzene deposited fine needles, m.p. 259°, proved to be identical with pyrid-[3,4-b]-indole-1(2)-one by mixed melting point and analysis. (Found: C, 71·64; H, 4·45; N, 15·01. Calc. for $C_{11}H_8N_2O$: C, 71·73; H, 4·34; N, 15·21%.)

Isolation of indole-2-carboxylic acid. The aqueous alkaline solution left after the removal of basic and neutral fractions from alkali fusion products was acidified and extracted with 200 cm³ ether. The ether extract on being washed with water, dried and distilled left a semi-solid residue. It was heated *in vacuo* at 0·01 mm when fine needles sublimed at 80–110°. This was further purified by several crystallizations from a mixture of petroleum ether (B.P. 40–60°) and ether and finally from benzene when shining flakes m.p. 199–200° were obtained (40 mg). (Found: C, 67·12; H, 4·45; N, 8·82. Calc. for $C_9H_7NO_2$: C, 67·08; H, 4·34; N, 8·69%.) The acid did not depress the m.p. of an authentic sample of indole-2-carboxylic acid when mixed.

Isolation of rhetsine and lupeol from fractions (A) and (B). The yellow crude solid (36 g) obtained from fractions (A) and (B) (*vide* p. 4) was dissolved in a large volume of ethanol by refluxing. The alcoholic solution on being concentrated and cooled deposited rhetsine. This was purified by several crystallizations from a mixture of chloroform and ethanol (1 : 2) when shining colourless rectangular plates, m.p. 270–271° (dec), $[\alpha]_D^{25°} = \pm 0°$ were obtained (0·9 g). It was sparingly soluble in ethanol, acetone, benzene, ether, ethyl acetate and methanol and insoluble in dilute acids and alkalies. (Found: C, 75·25; H, 5·53; N, 13·88; (N)—CH_3, 5·00; H, 0·30; mol. wt. 305. Calc. for $C_{19}H_{17}N_3O$: C, 75·24; H, 5·6; N, 13·86; (N)—CH_3, 4·91; H^+, 0·33%; mol. wt. 303.)

The alcoholic mother liquor left after separation of rhetsine was concentrated to a small volume to obtain crude lupeol (21 g). It was further purified by chromatography over alumina and subsequent crystallization from benzene and alcohol. The analytical sample melted at 212–213° and responded to Lieberman Burchard test. (Found: C, 84·50; H, 11·93. Calc. for $C_{30}H_{50}O$: C, 84·50; H, 11·73%.) Lupeol produced a crystalline acetyl derivative, m.p. 216–217°. Both lupeol and its acetate did not depress the m.p. of an authentic sample of lupeol and its acetyl derivative.

Chelerythrine. Chelerythrine was prepared by addition of sodium carbonate to an aqueous solution (100 cm³) of the chelerythrine chloride (C), (*vide* p. 5). From ethanol and methanol it formed very pale yellow leaflets, m.p. 210° (300 mg). (Found: C, 69·46; H, 5·60; N, 3·89; —OCH_3, 17·20. Calc. for $C_{21}H_{19}NO_5$: C, 69·10; H, 5·20; N, 3·83; —OCH_3, 16·90%.) It gave positive Labat test.

Chelerythrine chloride. 100 mg chelerythrine was dissolved in 10 cm³ 2 N HCl solution upon warming. On cooling golden-orange needles of chelerythrine chloride, m.p. 203–206° separated out. Repeated crystallizations from ethanol raised its melting point to 210° (dec). (Found: C, 65·48; H, 4·82; N, 3·76. Calc. for $C_{21}H_{18}NO_4Cl$: C, 65·71; H, 4·68; N, 3·65%.)

Chelerythrine picrate. The picrate was prepared by adding a saturated solution of aqueous picric acid to an aqueous solution (20 cm³) of the hydrochloride (50 mg). The yellow picrate separated crystallized from ethanol in fine needles, m.p. 236° (dec). (Found: N, 9·81. Calc. for $C_{27}H_{20}N_4O_{11}$: N, 9·72%.)

Deoxychelerythrine. 100 mg of chelerythrine chloride was dissolved in 50 cm³ methanol in which 1·0 g of sodium borohydride was added and left overnight. The solution gradually turned pale yellow and became colourless. The methanolic solution was freed from the solvent and the residue was treated with 25 cm³ water and shaken with chloroform. The chloroform layer was washed with water, dried over anhydrous sodium sulphate and distilled. The residue crystallized from ethanol in colourless needles, m.p. 165–166°. (Found: C, 72·11; H, 5·31; N, 4·14. Calc. for $C_{21}H_{19}NO_4$: C, 72·20; H, 5·4, N, 4·01%.)

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