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A GENERAL METHOD FOR THE ISOLATION OF NATURALLY OCCURRING WATER-SOLUBLE BASES

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Abstract—A gradient pH separation of the reineckates of water-soluble alkaloids, such as quaternary amines, betaines, and tertiary amine oxides, has been developed. Fourteen bases, representing six broad structural types, have been isolated from ten species belonging to the families *Leguminosae* and *Gramineae*. Several unidentified indole and other minor basic components were also detected by paper chromatography. A new spraying reagent, α-nitroso-β-naphthol, has been used for detecting hydroxy-β-phenylethylamines and for differentiating them from the hydroxytryptamines. The alkaloid isolation technique appears to be of general applicability and particularly suitable for removing choline, the common cholinergic principle, from medicinal plants prior to their pharmacological screening.

INTEREST in the present investigation stems from reports of the widespread natural occurrence of quaternary amines[1-3] and tertiary amine oxides,[3,4] their plausible role in alkaloid biogenesis,[5-7] and their physiological activities as neuromuscular blocking agents[3,8,9] and reported value in chemotherapy.[4] These two classes of compounds may not always be isolable from plant extracts due, generally, to their extreme water-solubility. Further, the usual method of their isolation through the formation of reineckates or Meyer's complexes is not straight forward especially when a mixture of these compounds is present. The silver sulphate and sulphuretted hydrogen methods of regeneration of bases from the reineckate and Meyer's complexes, respectively, also need modification.

During a study of alkaloidal reineckates, we have developed a fairly satisfactory method for the isolation and purification of several complex mixtures of water-soluble alkaloids using gradient pH separation of the reineckate complexes. The details of this method and the identification of the alkaloids are described in this paper.

RESULTS

Fourteen water-soluble bases representing six broad structural types, viz. β-carboline, indole-3-alkylamine, β-phenylethylamine, pyridine, pyrrolidine, and aliphatic amines, were

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isolated from ten species belonging to the families *Leguminosae* and *Gramineae*. In addition, a number of unidentified minor bases were also detected on paper chromatograms. The chloroform-soluble secondary and tertiary alkaloids isolated from some of these species were previously reported.[3, 6, 8–11] The water-soluble alkaloids isolated in the present investigation, their respective plant sources, and the parts of the plant investigated are given in Table 1.

TABLE 1. WATER-SOLUBLE ALKALOIDS

Alkaloid	Part of plant investigated*	Species (family)
N,N-Dimethyltryptamine-N_b-oxide	R, Ls, S	*Desmodium pulchellum* Benth. ex Baker (*Leguminosae*: *Papilionaceae*)
	Ls, S	*D. gangeticum* DC.
	Ls	*D. gyrans* DC.
	B	*Acacia caesia* Willd. (*Leguminosae*: *Mimosae*)
	Ls, S	*Mucuna pruriens* DC. (*Leguminosae*: *Papilionaceae*)
	Ls, F	*Arundo donax* L. (*Gramineae*)
5-Methoxy-*N,N*-dimethyltryptamine-N_b-oxide	Ls	*D. pulchellum*
2-Methyl-6-methoxy-β-carbolinium cation	S	*D. pulchellum*
	S	*D. gangeticum*
Hypaphorine	R	*D. gangeticum*
	B, Ls, S	*Erythrina variegata* L. (*Leguminosae*: *Papilionaceae*)
	R, Ls, S	*Abrus precatorius* L. (*Leguminosae*: *Papilionaceae*)
Hypaphorine methyl ester	S	*A. precatorius* L. (*Leguminosae*: *Papilionaceae*)
Candicine	R	*D. gangeticum*
Bufotenidine	Rh	*Arundo donax*
Dehydrobufotenine	Rh	*A. donax*
Gramine-N_b-oxide	Ls, F	*A. donax*
Gramine methohydroxide	F	*A. donax*
Trigonelline	R, Ls, S	*Abrus precatorius*
	Ls, S, P	*Cassia occidentalis* Linn. (*Leguminosae*: *Caesalpiniaceae*)
	Ls, P	*C. tora*
	R, Ls	*Ulex europaeus* Gorse (*Leguminosae*: *Papilionaceae*)
L-Stachydrine	Ls, P	*C. occidentalis*; *C. tora*
Choline	R	*D. gangeticum*
	R, Ls, P	*C. occidentalis*; *C. tora*
	R, Ls	*U. europaeus*
	Ls, S	*M. pruriens*
Betaine	Ls, S, P	*C. occidentalis*; *C. tora*
	R, Ls	*U. europaeus*
Unidentified bases	—	*D. gangeticum*; *M. pruriens*; *Arundo donax*; *C. occidentalis*

* R, roots; Ls, leaves and stems; B, bark; S, seeds; F, flowers; Rh, rhizomes; P, pods.

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DISCUSSION

It is well known[12] that the formation of alkaloid reineckates in aqueous solutions depends on the concentration of the conjugate acid form of the base and thus depends on the base strength (pK_a) of the alkaloid and the pH of the medium. In case of a quaternary ammonium compound (almost completely dissociated), precipitation of the reineckate complex can take place even in alkaline medium. The complex formation with betaines (pK_a 7–11) and *tert*amineoxides (pK_a 3–5) would, however, require neutral-acidic and acidic media, respectively.

The above principle formed the basis of separation of the three categories of alkaloids in the present investigation. The bases were subsequently regenerated from the reineckate complexes by passing their acetone or alcoholic solutions over a strong anion-exchange resin, viz. De-Acidite FF (pH 8).[10] A further gradient pH separation of the reineckate complexes was done where there were more than one basic component present in each category. McIlvaine's buffer in the pH range 2–7 was used in the latter case.

The course of separation of the individual bases was followed using paper chromatography. Besides Dragendorff and Ehrlich reagents, a new colour reagent, α-nitroso-β-naphthol-nitrous acid, was used for the detection of hydroxytryptamines and the hydroxy-β-phenylethylamines. The test was found to be very sensitive with *p*-hydroxy-β-phenylethylamines and only 5–7γ concentration of the base was required to produce a reddish purple colour. The corresponding *p*-methoxy compounds did not respond to this reagent. The reagent was previously used by Udenfriend *et al.*[13] for detecting hydroxyindoles. In the present investigation, the spray also served to distinguish *p*-hydroxy-β-phenylethylamines from hydroxytryptamines since the reddish purple colour produced by the former turned yellow on keeping while the violet colour produced by the latter remained stable for a long time.

The method adopted here can be of general applicability. Particularly significant is its suitability for isolating water-soluble basic active principles from medicinal plants and for removing the common interfering plant constituent, choline, prior to their pharmacological evaluation.

EXPERIMENTAL PROCEDURE

The isolation of single components, occurring in different parts of the plant species, was straight forward. In these cases, irrespective of the base strengths, the aqueous mother liquors, from the separation of $CHCl_3$-soluble secondary and tertiary bases, were acidified and the acidic solutions were treated with saturated aqueous solutions of ammonium reineckate. The dry reineckate complexes were taken in acetone or ethanol and were passed through De-Acidite FF resin column (pH 8).[10] Elutions were carried out with the same solvent. 5-ml fractions were collected. First three fractions, on evaporation, afforded a single basic entity. The mixed components were separated as described below for *Desmodium gangeticum*, *Cassia occidentalis*, and *Arundo donax*. The course of separation of the mixed alkaloids was followed through paper chromatography on Whatmann No. 1 paper using *n*-butyl acetate–*n*-butanol–acetic acid–water (85:15:40:22) as the developer. Three spraying reagents, viz. Dragendorff (D), Ehrlich (E), and α-nitroso-β-naphthol-nitrous acid (N)[13] were used. The identification of the crystalline compounds was by mixed m.p. determinations and by comparison of u.v. (EtOH), i.r. (Nujol), and mass spectra with authentic samples.

Isolation of Candicine, Choline, Hypaphorine, and N,N-*Dimethyltryptamine*-N_b-*oxide from the Roots of* Desmodium gangeticum *DC*

Dried and milled roots (*ca.* 2 kg) were defatted with petroleum (60–80°) and then hot extracted with EtOH for 16 hr. The alcoholic extract was processed in the usual way for $CHCl_3$-soluble primary, secondary, and tertiary bases. The aqueous alkaline mother liquor showed six Dragendorff-positive spots on paper

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chromatograms, R_f 0·18 (E and N, negative); 0·22 (E, blue; N, violet); 0·36 (E, negative; N, reddish purple changing to yellow); 0·44 (E, blue; N, violet); 0·51 (E, purple changing to blue; N, dull brown); and 0·88 (E, cherry-red; N, dull brown). The aqueous solution was treated with a saturated aqueous solution of ammonium reineckate, the light reineckate complex (2·8 g) was taken in acetone, and was passed through a column of De-Acidite FF (pH, 8). First three fractions of the eluates on evaporation afforded a brown basic gum (0·55 g) containing two components, R_f 0·18 and 0·36 (area of major intensity). A small portion of the base (*ca.* 0·1 g) was taken in conc. Na_2CO_3 (pH, ~12) and ammonium reineckate solution was again added to it when reineckate complex of the minor component, R_f 0·18, was preferentially precipitated.

Choline. The base regenerated from the latter complex was directly converted to the picrate. Choline picrate crystallized from aqueous EtOH as yellow fine needles, m.p. and mixed m.p. 240–242°. The identity of the base was further established by co-chromatography with authentic choline chloride, R_f 0·18.

Candicine. The major portion of the base (*ca.* 0·45 g), obtained above, was treated with an aqueous alcoholic KI when candicine iodide separated (0·28 g). The iodide crystallized from EtOH as colourless needles, m.p. 229–230° (lit.[14] m.p. 229–230°); R_f 0·36; λ_{max} 222, 277, and 300–305 (sh) nm. Superimposable i.r. spectrum with hordenine methiodide. (Found: N, 4·3. Calc. for $C_{11}H_{18}NOI$: N, 4·5%.)

The aqueous alkaline mother liquor from the separation of choline and candicine was neutralized (pH 6–7), some more ammonium reineckate solution was added to it, and the solution kept at room temperature for 4 hr. The brownish pink reineckate complex (1·8 g), m.p. 257–259°, was decomposed as described above.

Hypaphorine. The alkaloid, obtained above, crystallized from alcohol–acetone as colourless needles, m.p. 260–261° (lit.[15] m.p. 235° and 256°); R_f 0·51; u.v. λ_{max} 224, 275 and 292 nm; significant i.r. peak at λ 6·15 μ (CO^- of betaine); exhibited superimposable i.r. spectrum with authentic hypaphorine. The hydrochloride crystallized from EtOH as colourless needles, m.p. 232° (lit[15] m.p. 231–232°); i.r. λ_{max} 5·8 μ (CO_2H). (Found: C, 59·8; H, 6·5; N, 9·7. Calc. for $C_{14}H_{19}N_2O_2Cl$: C, 59·4; H, 6·7; N, 9·9%.)

The neutral aqueous mother liquor was finally acidified (pH, ~1) with H_2SO_4 and ammonium reineckate solution was again added to it to ensure complete precipitation of the residual water-soluble alkaloid. The dirty brown reineckate complex (0·5 g) was decomposed as described above.

N,N-*Dimethyltryptamine*-N_b-*oxide.* The basic oil (0·07 g), obtained above, exhibited R_f 0·88 and λ_{max} 224, 277, 288, and 292 nm. It was directly converted to the picrate and the crude picrate crystallized from methanol as yellow needles, m.p. and mixed m.p. 176–178°; co-chromatography with an authentic specimen showed a single spot at R_f 0·88.

Isolation of Choline, Trigonelline, L-*Stachydrine, and Betaine from the Leaves and Stems of* Cassia occidentalis

The fresh leaves and stems of *C. occidentalis* (*ca.* 2 kg) were macerated in a Waring blender covered with N HCl (1 l.) and stirred for 12 hr at room temperature. An aliquot (200 ml) of the clarified acidic extract was basified (pH 9) and saturated ammonium reineckate was added to it when choline reineckate separated as pink needles. It was decomposed and the base was identified as described above.

The aqueous alkaline mother liquor was acidified (pH ~5) and some more ammonium reineckate solution was added to it when another crop of the reineckate complex precipitated (4·3 g). The regenerated base showed three Dragendorff-positive spots of major intensities at R_f 0·22, 0·28 and 0·33 plus three other faint spots at R_f 0·41, 0·43 and 0·49. None of these components responded to Ehrlich or α-nitroso-β-naphthol–nitrous acid reagent.

Trigonelline (as the gallate). To a portion of the above base methanolic solution of gallic acid was added and the solution was concentrated. The brown amorphous trigonelline gallate crystallized from methanol as cream-coloured plates, m.p. 208° (lit.[3] m.p. 208°); R_f 0·14; u.v. λ_{max} 210–216, 268 and 288 nm; i.r. λ_{max} 2·8 (OH), 5·4–5·5 μ (CO_2H of nicotinic acid); and the corresponding base showed R_f 0·33. (Found: C, 54·4; H, 4·3; N, 4·2. Calc. for $C_7H_8NO_2$,$C_7H_5O_5$: C, 54·7; H, 4·2; N, 4·5%.)

The weakly acidic mother liquor from the separation of trigonelline reineckate was strongly acidified (pH < 1), some more reineckate solution was added to it, and the mixture kept at room temperature for 4 hr. The pink reineckate complex (1·8 g) was decomposed. The base showed two major spots at R_f 0·22 and 0·28 plus a faint trailing from 0·3–0·5. It was taken in alcohol and picric acid solution in the same solvent was added to it. The picrate crystallized from alcohol as orange needles, m.p. 188–189°.

Betaine. The identity of the above picrate to betaine picrate was established by mixed m.p. 188–189° and co-chromatography of the base with an authentic specimen, R_f 0·28, which showed a single spot at the same R_f value.

L-*Stachydrine.* The alcoholic mother liquor from the separation of betaine picrate was again passed through De-Acidite FF column. The regenerated base, obtained as a syrupy liquid (0·12 g), R_f 0·22, crystallized from absolute alcohol as hygroscopic needles, m.p. and mixed m.p. 112–114° (lit.[16] m.p. 116–118°); co-chromatography with an authentic specimen showed a single spot having the same R_f value.

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Isolation of Bufotenidine, Dehydrobufotenine, and Gramine-N_b-oxide from the Rhizomes of Arundo Donax

The aqueous alkaline mother liquor from the separation of the $CHCl_2$-soluble secondary and tertiary bases,[8] was treated with a saturated aqueous solution of ammonium reineckate. The amorphous complex (5·2 g) was decomposed over De-Acidite FF column.

Bufotenidine. The regenerated base showed two major spots at R_f 0·06 (D, orange; E, negative; N, violet) and 0·18 (D, orange; E, blue; N, violet). It was taken in EtOH and was treated with picric acid solution in the same solvent. The reddish yellow picrate crystallized from aqueous alcohol as orange needles, m.p. 198–200° (lit.[17] m.p. 198°). Mixed m.p. with the picrate from authentic bufotenine methiodide, m.p. 198–200° remained undepressed. (Found: N, 15·8. Calc. for $C_{13}H_{18}N_2O$, $C_6H_3N_3O_7$: N, 15·6%.)

Dehydrobufotenine. The aqueous alkaline mother liquor from the separation of bufotenidine reineckate was neutralized (pH 6–7), some more ammonium reineckate solution was added to it, and the mixture was kept at room temperature for 4 hr. The second crop of the complex was decomposed over the resin column and the regenerated base (0·15 g), R_f 0·06, was directly converted to the picrate. The picrate crystallized from water as yellow needles, m.p. 182–184° (lit.[17] m.p. 183–184°). (Found: N, 15·9. Calc. for $C_{12}H_{14}N_2O$, $C_6H_3N_3O_7$: N, 16·2%.)

The aqueous neutral mother liquor, obtained above, was acidified (pH < 1) and the solution, on keeping at room temperature for 4 hr, afforded a third crop of the reineckate complex (2·7 g). The base was regenerated from the complex in the usual way.

Gramine-N_b-oxide. The regenerated base (0·51 g) showed four spots at R_f 0·08 (D, orange; E, negative; N, violet); 0·17 (D, orange; E, blue; N, violet); 0·23 (D, orange; E, purple; N, violet); and 0·92 (D, brownish orange; E, cherry-red; N, dull-brown). A few drops of ethanolic H_2O_2 was added and the mixture was kept at room temperature for 3–4 days. Gramine-N_b-oxide, H_2O_2 adduct separated as plates, m.p. 120–122° (lit.[18] m.p. 122°); R_f 0·91; λ_{max} 220, 270 and 288 nm. Zn dust-acetic acid reduction of the N_b-oxide afforded gramine, crystallized from benzene as flakes, m.p. and mixed m.p. 133°.

The mother liquor from the separation of gramine-N_b-oxide was evaporated to dryness. The residue (0·29 g) was taken in water and treated with ammonium reineckate solution at three pH levels, viz. 6·4, 2·9 and < 1. This afforded a further crop of dehydrobufotenine (pH 6·4); R_f 0·08; and the two unidentified indole bases, R_f 0·23 (pH 2·9); λ_{max} 222, 272, 288 and 305 (sh) nm; and R_f 0·16 (pH < 1); λ_{max} 224–226, 275, 290 and 305–310 nm.

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