DITERPENE ALKALOIDS. ISOLATION AND STUDY OF TWO NEW ALKALOIDS

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Two new alkaloids tentatively named as "denudatine" and "denudatidine" have been isolated in pure form from an indigenous crude drug "Judwar" which has been provisionally identified as the roots of *Delphinium denudatum* Wall. (Fam. Ranunculaceae). The purity of these alkaloids has been checked by paper chromatographic analysis and they have been characterised. Denudatine is the major alkaloid and has been isolated in pure form in a yield of 0·05 per cent. On the basis of microanalysis for the elements of the base, m.p. 248–249° and its derivatives; the determination of the active hydrogen, =N–Me, ≡C–Me and infra-red and ultra-violet absorption spectra, denudatine has been tentatively classified as a diterpene alkaloid of atisine group. Pharmacological actions of denudatine have been investigated. The normal rhythmic contractions of isolated rabbit duodenal strip are inhibited. The isolated guinea-pig uterine strip is stimulated by the base solution. It decreases the tone and inhibits the peristaltic movements of the intestine but does not show any effect on the blood pressure and respiration of the anaesthetised dog. It is non-toxic and lacks curariform activity. Denudatidine, m.p. 273° (yield, 0·003 per cent) has not been studied further.

The tuberous roots of a large number of *Aconite* and *Delphinium* species are exploited commercially in India and have been used in the indigenous system of medicine for treating numerous ailments. The Indian species of these plants have largely remained uninvestigated except for the preliminary reports on chemical investigations by Dunstan and others (c.f. Stern, 1954, 1960; Henry, 1949; Chopra, Chopra, Handa and Kapur, 1958), although considerable interest has been shown by foreign workers in the elucidation of the structures of the diterpene alkaloids present in the various locally available species (Stern, 1954, 1960; Wiesner and Valenta, 1958). We have undertaken to investigate commercial samples with the object of elucidating the structures of the alkaloids present.

Herein we report the results of a preliminary study of a root "Judwar" which has been provisionally identified as the root of *Delphinium denudatum*. Wall. (Fam. Ranunculaceae). The crude drug has reported uses in some diseases of the blood, in insanity and as stimulant in conditions of debility. It is also used against painful piles and toothache, and as antidote to snake and scorpion venoms (Kirtikar and Basu, 1933). No previous investigation of any part of this plant has been reported.

Experimental

Materials

The tuberous roots were obtained from the Amritsar crude drug market under the name "Judwar" (it is also known as "Nirbishi"). Roots were

conical, dark brown to blackish in colour, 1·5 to 7·0 cm. long, 1 to 2·5 cm. in diameter at the crown, occurring largely singly. Internally the root was yellowish to dark brown marked by a central light portion with an angular line representing cambium. The taste was initially bitter and was then followed by sensation of numbness. The root has been provisionally identified by Mr. S. N. Sobti of the Regional Drug Research Laboratories, Jammu (India).

Extraction and Isolation of Denudatine and Denudatidine

Root powder (7 kg.) was extracted with ethanol (95 per cent) in a soxhlet apparatus (liming the drug before extraction did not improve the yield or facilitate extraction) until exhausted of alkaloids (8 hr.). The ethanol was recovered from the extract and the dark viscous residue was dissolved in hydrochloric acid (5 per cent) and the acid liquor extracted with ether (10 × 35 ml.) to remove non-basic impurities. The insoluble residue that separated at the interface was removed and washed free of alkaloids with dilute acid and the acid washings were added to the acid liquor. The acid liquor was made alkaline with ammonia solution and alkaloid extracted with ether (18 × 45 ml.). The ether solution was dried over exsiccated sodium sulphate and the ether was removed to yield a residue (17·6 g.), which was again dissolved in hydrochloric acid (60 ml. 5 per cent), made alkaline with ammonia solution and extracted with ether (15 × 35 ml.). The solvent was removed to yield a crude base "A" (12·9 g.). The crude base "A" was purified by washing the resinous colouring impurities with ethanol (10 ml. 60 per cent) and recrystallisation from benzene (or absolute ethanol) to yield light white needles (3·5 g. 0·05 per cent), m.p. 246–47°. Two recrystallisations raised the m.p. to 248–49°. The base was provisionally named "denudatine" and was proved to be a single chemical entity by paper chromatography.

The alkaline liquor left after extraction of base "A" with ether, was extracted with chloroform (15 × 30 ml.). The chloroform solution was dried over exsiccated sodium sulphate, the solvent removed and the residue (4·9 g.) dissolved in hydrochloric acid (30 ml. 5 per cent), made alkaline with ammonia solution and extracted with chloroform (15 × 20 ml.). The solvent was dried and removed to yield a residue (1·3 g.) of crude base "B", which was purified by adsorption on an alumina (Merck) column from acetone solution and eluting it with the same solvent, followed by crystallisation from absolute ethanol to yield shining light brown prismatic crystals (0·2 g., 0·003 per cent), m.p. 273°. This base was provisionally named "denudatidine" and was found to be a single chemical entity by paper chromatographic analysis.

Paper Chromatographic Analysis

The ascending strip paper and circular paper methods were employed. Whatman No. 1 filter paper buffered at pH 4·0 by sodium dihydrogen-citrate was used. The solvent system consisted of butanol : water : citric acid (50 : 50 : 1). The organic layer was employed as the mobile phase. Samples for analysis consisted of the ether and the chloroform residues,

the crude bases "A" and "B" and the pure bases, denudatine and denudatidine. Development was at 20° for 6–7 hr. The chromatograms were air-dried and dipped in Munnier's modified Dragendorff's reagent (Block, Le Strange and Zweig, 1952) and R_F values were calculated. Denudatine and denudatidine were the only two bases indicated to be present in the root with their average R_F values of 0·80 and 0·63 respectively. Both alkaloids gave orange spots on a yellow background.

Chemical Characterisation of Denudatine. Analyses by Drs. Weiler and Straus

Denudatine was very soluble in absolute ethanol; slightly less soluble in methanol, benzene and carbon disulphide; sparingly soluble in chloroform, ethyl acetate and dioxan; practically insoluble in water and carbon tetrachloride. The base solution in hydrochloric acid (1 per cent) gave positive tests with most of the precipitating alkaloidal reagents. Found: C, 75·7; H, 10·0; N, 4·3 per cent; Mol. wt. 294 (Cryoscopic method in benzene); $[\alpha]_D^{21}$ (Ethanol) = +0·154. Calc. for $C_{21}H_{33}NO_2$: C, 76·1; H, 10·0; N, 4·2 per cent. Mol. wt., 331.

Denudatine hydriodide. This was prepared by adding potassium iodide solution (10 per cent) in a slight excess to a saturated solution of the base in dilute hydrochloric acid and recrystallisation of the precipitate produced from ethanol (60 per cent), m.p. 267–268°. Found: C, 54·4; H, 7·5; I, 28·0; N, 3·2 per cent. Calc. for $C_{21}H_{33}NO_2HI$: C, 54·9; H, 7·4; I, 27·7; N, 3·2 per cent.

Denudatine picrate. This was prepared by adding a saturated solution of picric acid in water in a slight excess to a saturated solution of the base in dilute hydrochloric acid and recrystallisation of the yellow precipitate from methanol, m.p. 215°. Found: C, 57·5; H, 5·8; N, 9·8 per cent. Calc. for $C_{27}H_{35}N_4O_9$: C, 57·9; H, 6·0; N, 10·0 per cent.

Denudatine-gold chloride complex was prepared by adding gold chloride solution (2 per cent w/v) to a saturated solution of the base in dilute hydrochloric acid and recrystallisation of the product from alcohol or acetone, m.p. 158–59°. Found: C, 40·3; H, 5·0; N, 2·4 per cent. Calc. for $C_{21}H_{33}NO_2.AuCl_3$: C, 39·7; H, 5·2; N, 2·2 per cent.

Denudatine reineckate was prepared by adding ammonium reineckate solution (1 per cent w/v) to a solution of the base in dilute hydrochloric acid as microcrystalline power, m.p. 202–203°. Found: C, 44·3; H, 6·4; N, 13·7 per cent. Calc. for $C_{25}H_{40}N_7O_2S_4$ $Cr.2H_2O$: C, 43·7; H, 6·4; N, 14·3 per cent.

Diacetyldenudatine. Denudatine (100 mg.), dry pyridine (1 ml.) and acetic anhydride (0·2 ml.) were refluxed together on a sand bath for 4 hr., cooled, poured into water (25 ml.) and ammonia solution was added to precipitate the product. It was separated and recrystallised from absolute ethanol as microcrystalline powder, m.p. 128–30°. Found: C, 72·3; H, 8·7 per cent. Calc. for $C_{21}H_{31}NO_2(COMe)_2$: C, 72·3; H, 8·9 per cent.

Trichloroacetyl denudatine was prepared by heating a saturated aqueous solution of trichloroacetic acid with a saturated solution of the base in

dilute hydrochloric acid solution (1 per cent) and recrystallisation from absolute ethanol, m.p. 235°. Found: C, 46·5; H, 4·8; N, 2·0 per cent. Calc. for $C_{21}H_{31}NO_2(COCCl_3)_2.H_2O$: C, 46·9; H, 5·2; N, 2·2 per cent.

Active hydrogen. Found: 0·5 per cent. Calc. for 2 active hydrogens: 0·6 per cent.

Tertiary carbon-methyl group. Found: 5·7 per cent. Calc. for one ≡ C–Me: 8·2 per cent.

N-*Methyl group.* On heating the base with soda-lime, vapours having an ammoniacal odour were evolved and when these were passed into a solution of hydrochloric acid in ether, a precipitate was formed, which on recrystallisation melted at 225° and was identified as methylamine hydrochloride by mixed melting point determination. On treating the base

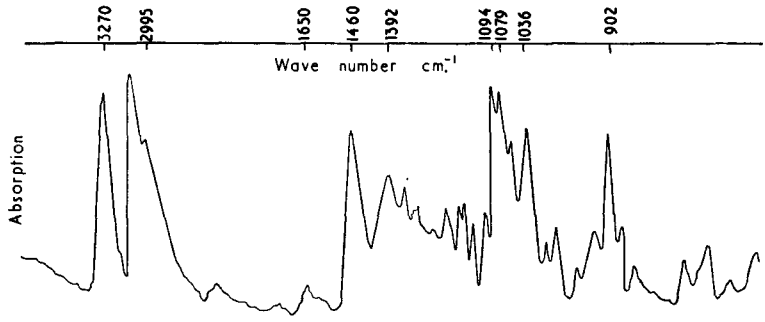

FIG. 1. Infra-red spectrum of denudatine.

with ice-cold nitrous acid, a gas was evolved, but it did not give Liebermann's test and a dye test, Rimnis' test and the carbon-disulphide reagent test were also negative. Found: 8·7 per cent. Calc. for one = *N*–Me; 8·8 per cent.

Ultra-violet spectrum. The ultra-violet spectrum was determined on a DU-spectrophotometer (Beckman) equipped with fused silica prisms. Denudatine in methanol (0·0045 per cent) exhibited only one absorption maximum at 210 mμ. Molar extinction coefficient ϵ = 4,925.

Infra-red spectrum. The infra-red spectrum of denudatine in Nujol indicated the presence of hydroxyl group (3,270 cm.$^{-1}$), i.e., vibration frequency of H–O bond supported by the vibration frequencies at 1,094 and 1,079 cm.$^{-1}$ due to C–O bond; exomethylene group, C=CH_2 (2,995 cm.$^{-1}$, C–H stretching frequency as shoulder on the Nujol band; 1,650 cm.$^{-1}$, C=C stretching frequency and 902 cm.$^{-1}$, C–H deformation frequency; =C–Me (C–H deformation frequencies at 1,460, 1,392 and 1,368 cm.$^{-1}$) (Fig. 1).

These groups were confirmed by the infra-red spectra of the base hydriodide in KCl mulls done on a Grubb-Parsons infra-red spectrophotometer. OH (3,436, 1,092, 1,075 cm.$^{-1}$); N–Me (2,900 cm.$^{-1}$); C=CH_2 (2,941, 1,653, 905 cm.$^{-1}$); C–Me (1,481, 1,449, 1,394 and 1,370 cm.$^{-1}$). There was no evidence for the presence of a carbonyl group or a lactam

group. It further indicated that the salt was not anhydronium salt and thus denudatine is not a carbinol base nor is the base a carbinolamine.

Chemical Characterisation of Denudatidine

Denudatidine was found to be soluble in ethanol, methanol, acetone, pyridine, and dioxan while sparingly soluble in ethyl acetate, light petroleum and carbon tetrachloride. The base solution in hydrochloric acid (1 per cent) gave positive tests with all the general precipitating alkaloidal reagents. Found: C, 67·6; H, 8·6; N, 3·3 per cent; Titration equivalent 400 ± 20 (Electrometric method); $[\alpha]_D^{21}$ (ethanol) = +31·56. Calc. for $C_{23}H_{35}NO_5$: C, 68·1; H, 8·6; N, 3·5 per cent; Mol. wt. 405; for $C_{23}H_{37}NO_5$: C, 67·8; H, 9·1; N, 3·4 per cent.

Denudatidine reineckate was prepared by adding ammonium reineckate solution (2 per cent) to a saturated solution of the base in hydrochloric acid (1 per cent). The reineckate was purified by passing its solution in acetone through an alumina column; it was then crystallised from acetone as microprisms, m.p. 190°. Found: C, 44·3; H, 6·0; N, 13·4 per cent. Calc. for $C_{27}H_{42}CrN_5O_5S_4$: C, 44·8; H, 5·8; N, 13·5 per cent; for $C_{27}H_{44}CrN_5O_5S_4$: C, 44·6; H, 6·1; N, 13·5 per cent.

Denudatidine picrate was prepared by adding a saturated ethanolic solution of picric acid to a saturated solution of the base in absolute ethanol; the product was recrystallised from methanol as yellow microneedles, m.p. 100°. Found: C, 51·0; H, 6·1 per cent. Calc. for $C_{29}H_{40}N_4O_{12}$: C, 52·0; H, 6·6 per cent.

PHARMACOLOGICAL

Blood pressure and respiration studies were made on healthy dogs (2–4 kg.) anaesthetised with phenobarbitone (150 mg./kg. i.p.). The effects were recorded by direct cannulation of the carotid artery and trachea respectively. Denudatine was administered through the femoral vein. With a dose of 0·5 to 5 mg./kg., blood pressure and respiration remained unaffected.

The alkaloid given at a dose of 0·5–5 mg./kg. produced a decrease of tone and inhibition of peristaltic movements of the dog intestine *in situ*. At a concentration of 2×10^{-5}M it produced a marked inhibitory effect on the movements of the rabbit isolated duodenal strip. The contractions induced by acetylcholine, 2×10^{-5}M, and by histamine, 8×10^{-6}M, in guinea-pig intestinal strips, were not antagonised by the alkaloid, 2×10^{-5} M. But at this concentration it stimulated isolated guinea-pig uterine strips. Atropinisation did not alter the effect of drug.

Curariform activity was determined in dogs anaesthetised with phenobarbitone. The gastrocnemius nerve-muscle preparation was set up according to the technique of Hoppe (1950) for observing the action of the drug on the neuromuscular transmission. Denudatine in doses of 200 mg./kg. given i.v., did not produce any curariform effect on the muscle.

For these studies, denudatine was dissolved in a mixture of ethanol, propyleneglycol and distilled water (1 : 1 : 2) and the mixture adjusted to

pH 7·2. In all experiments proportionate amounts of the solvent were given as controls.

Discussion

The microanalytical data of the alkaloid, denudatine and its salts and derivatives show that its molecular formula is $C_{21}H_{33}NO_2$. Both the oxygen atoms are present as alcoholic hydroxyl groups as confirmed by the infra-red spectra and active hydrogen determination. The nitrogen atom is tertiary and carries a methyl group. An exomethylene group is indicated by the infra-red spectra. The presence of a methyl group attached to a tertiary carbon atom is indicated by analysis. Denudatine is, therefore, most probably a diterpene alkaloid of atisine group with pentacyclic structure bearing one unsaturated linkage. The ultra-violet spectrum indicates that one of the two hydroxyl groups is an allylic alcoholic group in relation to an exomethylene group. Further work on the constitution of denudatine has been reported by Nazar Singh (1961). Se-dehydrogenation studies recently reported (Singh, Singh and Malik, 1961) confirm the constitution, 1-methyl-6-ethylphenanthrene and 1-methyl-6-ethyl-3-azaphenanthrene being obtained.

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