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ALKALOIDS OF *ACONITUM FEROX*

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Key Word Index—*Aconitum ferox*; Ranunculaceae; aconitine alkaloids, veratroyl pseudaconine; diacetyl pseudaconitine.

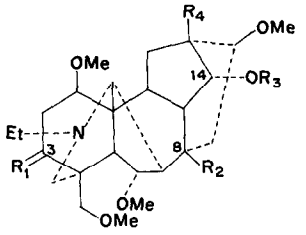
Abstract—From the roots of *Aconitum ferox* four alkaloids have been isolated. Pseudaconitine is the major constituent and the other three minor alkaloids are identified as bikhaconitine, veratroyl pseudaconine and diacetyl pseudaconitine. Veratroyl pseudaconine and diacetyl pseudaconitine have not been previously found in nature.

INTRODUCTION

PREVIOUS investigations[1,2] of *Aconitum ferox** have resulted only in the isolation of pseudaconitine (Alkaloid-B). In the present communication, the isolation of three more alkaloids (A, D and C) is reported. These separations have been achieved by a differential solvent extraction technique and careful pH-gradient fractionation of the basic components. The identities of these alkaloids have also been established.

RESULTS AND DISCUSSION

Both the alkaloid-A, $C_{36}H_{51}NO_{11}$ (M^+ 673), m.p. 118° and alkaloid B, $C_{36}H_{51}NO_{12}$ (M^+ 689), m.p. 208°, contain an iminoethyl group, hydroxyl groups, six methoxyls of which two are aromatic, acetoxy ester functions and an aryl ester carbonyl group. The alkaloids on alkaline hydrolysis gave veratric acid, acetic acid, and an alkamine in each case, the former yielding bikhaconine (**3**) and the latter pseudaconine (**4**), m.p. 90–92°. The NMR spectra of the two alkaloids were essentially similar to those reported for bikhaconitine (**1**) and pseudaconitine (**2**) respectively. Direct comparison of both the bases (m.p., m. m.p., TLC and superimposable IR spectra) with authentic specimens confirmed their identity.



(**1**) $R_1 = H_2$; R_2 = OAc; R_3 = Vr; R_4 = OH
(**2**) $R_1 = \alpha$OH, H; R_2 = OAc; R_3 = Vr; R_4 = OH
(**3**) $R_1 = H_2$; $R_2 = R_4$ = OH; R_3 = H
(**4**) $R_1 = \alpha$OH, H; $R_2 = R_4$ = OH; R_3 = H
(**5**) $R_1 = \alpha$OH, H; R_2 = OAc; R_3 = Bz; R_4 = OH
(**6**) $R_1 = \alpha$OAc, H; $R_2 = R_4$ = OAc; R_3 = COMe
(**7**) $R_1 = \alpha$OH, H; $R_2 = R_4$ = OH; R_3 = Vr
(**8**) $R_1 = H_2$; R_2 = OAc; R_3 = Bz; R_4 = OH
(**9**) $R_1 = \alpha$OAc, H; $R_2 = R_4$ = OAc; R_3 = Vr

Bz = Benzoyl ; Vr = Veratroyl

* The commercial drug obtained from Madras Market was carefully screened pharmacognostically in the department of botany of this Institute, to remove any extraneous materials.

[1] WRIGHT, C. R. A. and LUFF, A. P. (1878) *J. Chem. Soc.* **33**, 151.
[2] DUNSTAN, W. R. and CARR, F. H. (1897) *J. Chem. Soc.* **71**, 350.

The alkaloid D is one of the minor constituents of the alcoholic extract of *A. ferox*. The elemental analysis and the MW (M^+ 647) established its formula as $C_{34}H_{49}NO_{11}$. It contained one or more hydroxyl groups (broad band at 3380 cm^{-1}), an aromatic ring (1600 cm^{-1}) and an ester carbonyl function (1708 cm^{-1}). The NMR spectrum of the base indicated the presence of six methoxyls (δ3·22, 3·28, 3·30, 3·46 and 3·90) of which two were aromatic (δ3·90, 6H,*s*). There were also signals characteristic of an *N*-ethyl group (δ1·12, 3H,*t*) and of a 3,4-dimethoxybenzoyl (veratroyl) group [δ6·86, 1H, *d*, *J* 4·5 Hz (C-5 proton of veratroyl); δ7·61, 7·72 (C-2 and C-6 protons of the veratroyl group)]. The spectrum also exhibited a one proton doublet at δ5·1 (*J* 4·5 Hz) attributable to a proton (C-14) attached to a carbon carrying an aromatic ester group. Similar spectral behaviour has been observed in indaconitine[3] (**5**), pseudaconitine[4] (**2**) and bikhaconitine[5] (**1**) having an equatorial C-14 aryl ester function.

The alkaline hydrolysis of alkaloid D produced veratric acid, acetic acid and a crystalline alkamine (**4**), m.p. 90°, having four methoxyls. Treatment of this alkamine with acetic anhydride in *p*-toluene sulfonic acid gave a derivative $C_{33}H_{49}NO_{12}$, m.p. 226° which was identical in all respects (m.p., m. m.p., TLC and superimposable IR spectra) with pseudaconine tetracetate (**6**), prepared from authentic pseudaconitine by alkaline hydrolysis followed by a similar acetylation. Consequently the alkaloid D, should be veratroyl pseudaconine (**7**). This was confirmed by heating pseudaconitine with 0·1 N H_2SO_4 when veratroyl pseudaconine[6] was obtained and the product was found identical in all respects (m.p., m. m.p., TLC and superimposable IR spectra) with the product from *A. ferox*.

The alkaloid C, analysed for $C_{40}H_{55}NO_{14}$ (M^+ 773), and was obtained from the weak base fractions of the alcohol extract of *A. ferox*. The IR spectrum of the compound showed the presence of two ester carbonyls, one of which is associated with an aryl ester (1710 cm^{-1}) and the other with acetoxy ester groups (1725 and 1248 cm^{-1}). The compound, however, shows no hydroxyl absorption in the IR. In the NMR spectrum a triplet of 3H centered around δ1·08, characteristic of an *N*-ethyl, and six methoxyls (δ3·39, 3·24, 3·21 and 3·85) were present. The signal at δ3·85 (6H,*s*) is attributable to two aromatic methoxyls. Signals were also observed for three acetoxyl functions, two of them appearing as a six proton singlet at δ2·03 and the third one as a singlet of three protons at a higher field than usual (δ1·29). This upward shift has been observed earlier in the NMR spectra of indaconitine[3] (**5**) chasmaconitine[7] (**8**) and pseudaconitine[4] (**2**) and is caused by the protons of the acetoxyl group coming within the shielding range of the aromatic nucleus. Hence, location of this acetoxyl in alkaloid C must be at C-8. The spectrum further disclosed the presence of three aromatic protons characteristic of a veratroyl group [δ6·88, 1H, *d*, *J* 5 Hz; 7·63, 7·72; (C-5, C-2 and C-6 protons of veratroyl)] and a multiplet of two protons centred around δ4·81 (C-14 and C-3 protons respectively). Saponification of the alkaloid yielded veratric acid, acetic acid and an alkamine which on complete acetylation yielded pseudaconine tetracetate (**6**), m.p. and mixed m.p., 226°. Since the alkamine derived from Alkaloid C has been characterized as pseudaconine and also since the alkaloid had no OH group and contained 3 acetoxyls, it was concluded that the alkaloid in all probability was pseudaconitine diacetate (**9**). This was confirmed by conversion[6] of pseudaconitine to its diacetyl

[3] GILMAN, R. E. and MARION, L. (1962) *Tetrahedron Letters* 923.
[4] TSUDA, Y. and MARION, L. (1963) *Canadian J. Chem.* **41**, 1634.
[5] TSUDA, Y. and MARION, L. (1963) *Canadian J. Chem.* **41**, 3055.
[6] TSUDA, Y. and MARION, L. (1963) *Canadian J. Chem.* **41**, 1485.
[7] ACHMATOWICZ, O. Jr. and MARION, L. (1964) *Canadian J. Chem.* **42**, 154.

derivative. The reaction product was identical in all respects (m.p. m. m.p., TLC and superimposable IR spectra) with Alkaloid C.

The new compounds isolated have been correlated to pseudaconitine whose structure and stereochemistry has already been established.[6]

EXPERIMENTAL

All m.ps. are uncorrected. NMR spectra were recorded in $CDCl_3$ with TMS as internal standard on a 100 MHz instrument. The analytical samples were dried at 80° over P_2O_5 *in vacuo*. Brockman alumina was used for column chromatography.

Isolation of alkaloids. Dried ground root of *A. ferox* (10 kg) was exhaustively extracted successively with cold hexane and $CHCl_3$. The $CHCl_3$ extract (8·5 g) was digested with 3% HCl (3 × 150 ml) and the filtered acid soln extracted with CH_2Cl_2 to remove non-basic components. The aq. layer was made alkaline (pH 11) with NH_3 and exhaustively extracted with CH_2Cl_2 and dried. The oily concentrate (1·2 g) was chromatographed on a column using C_6H_6–$CHCl_3$ (1:6) from which alkaloid-A, bikhaconitine (**1**; 60 mg), was obtained as colourless stellate crystals, m.p. 118° (lit. m.p. 118–123°). (Found: C,62·36; H, 7·18; N, 2·13. $C_{36}H_{51}NO_{11}.H_2O$ requires C, 62·51; H, 7·38; N, 2·02%). The $CHCl_3$ eluates afforded alkaloid-B, pseudaconitine (**2**; 200 mg), m.p. 208° MS (M^+ 689) as colourless prisms from Me_2CO–hexane. (Found C, 62·61; H, 7·26; N, 2·30. $C_{36}H_{51}NO_{12}$ requires C, 62·69; H, 7·45; N, 2·03%). The marc was then extracted with EtOH, the extract conc. under red. pres. (3·62 g) and digested with 3% HCl. The aq. acid layer, after extraction with CH_2Cl_2, was adjusted to pH 8 with Na_2CO_3 and then exhaustively extracted with CH_2Cl_2. Removal of the solvent left a crude residue (500 mg; Extract I.) The aq. layer was then made strongly basic with NaOH (pH 12) and extracted further with CH_2Cl_2 to yield a mixture of strong bases (200 mg; Extract II). Column chromatography of Extract I failed to effect a clean separation but gave a fraction rich in alkaloid C. Separation was effected on this fraction by preparative TLC using EtOAc–MeOH (19:1). The material obtained from the plates was extracted with CH_2Cl_2 and the residue chromatographed using C_6H_6–$CHCl_3$ (1:1) as eluent. The resulting alkaloid C (**9**; 50 mg.) crystallized out in needles from Me_2CO–hexane mixture, m.p. 230° MS (M^+ 773). (Found: C, 62·55; H, 7·02, N, 1·68 $C_{40}H_{55}NO_{14}$ requires C, 62·09; H, 7·16; N 1·81%).

Extract II was also separated by preparative TLC using 5 plates (40 × 40 cm) and developed with $CHCl_3$–MeOH (9:1) mixture. The band of silica gel containing alkaloid D, was eluted on a short column using $CHCl_3$–MeOH (20:1) and colourless microcrystals of alkaloid D, m.p. 212° MS (M^+ 647), (**7**; 80 mg.) were obtained. (Found: C, 62·86; H, 7·21, N, 2·36. $C_{34}H_{49}NO_{11}$ requires C, 63·06; H, 7·57; N, 2·16%).

Pseudaconine. Pseudaconitine (150 mg) was hydrolysed by heating with 3% methanolic KOH (10 ml) for 2 hr on a steam bath. The reaction mixture on usual working up and crystallization from Me_2CO–MeOH yielded pseudaconine, m.p. 90–92° (**4**; 50 mg). Acidification of the aq. fraction and extraction with $CHCl_3$ gave veratric acid, m.p. 181°; the aq. soln contained HOAc.

Pseudaconine tetracetate. Pseudaconine (50 mg) was heated with Ac_2O (3 ml) and *p*-toluene sulphonic acid (50 mg) at 100° for 2 hr. The reaction mixture after the usual work-up left a gummy residue which was chromatographed. The C_6H_6–$CHCl_3$ (3:1) eluates afforded pseudaconine tetracetate (**6**; 30 mg.) m.p. 226° (Me_2CO-hexane). (lit. m.p. 228°). (Found: C, 60·98; H, 7·33; N, 2·04. $C_{33}H_{49}NO_{12}$ requires C, 60·83; H, 7·5; N, 2·15%).

Veratroyl pseudaconine from pseudaconitine. Pseudaconitine (50 mg) dissolved in 0·1 N H_2SO_4 (10–12 ml) was heated in a sealed tube at 140° for 5 hr and the contents made alkaline with 0·1 N KOH and extracted with $CHCl_3$. The crude base on chromatography yielded veratroyl pseudaconine (**7**; 30 mg) m.p. 212°.

Hydrolysis of veratroyl pseudaconine. Veratroyl pseudaconine (50 mg) was hydrolysed with 3% methanolic KOH (12 ml) and the reaction mixture on working up in the usual way afforded pseudaconine (**4**; 30 mg), m.p. 90° and veratric acid, m.p. 183°.

Hydrolysis of diacetyl pseudaconitine. The alkaline hydrolysis of diacetyl pseudaconitine was carried out as before. The following products viz. pseudaconine, veratric acid and acetic acid were characterized.

Preparation of diacetyl pseudaconitine. Pseudaconitine (30 mg) was heated with Ac_2O (3 ml) and p-toluene sulphonic acid (50 mg) at 100° for 2 hr. The reaction product was diluted, made alkaline and extracted with CH_2Cl_2. The crude base so obtained after chromatography gave diacetyl pseudaconitine (**9**; 25 mg), m.p. (MeOH–Et_2O) 228°.

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