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# THE ALKALOIDS OF *ACONITUM HETEROPHYLLUM* WALL.: ISOLATION AND CHARACTERIZATION

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**Abstract**—A detailed study of the basic components of the roots of *Aconitum heterophyllum* has led to the isolation of seven new diterpene alkaloids. The weak base fraction yielded besides heteratisine, three more alkaloids designated as *heterophyllisine*, *heterophylline*, and *heterophyllidine*. These compounds are lactone alkaloids which are structurally related to heteratisine. The strong base fraction yielded besides atisine two new alkaloids, *atidine* and *F-dihydroatisine*, the latter previously encountered as a reduction product of atisine and isoatisine. The very strong base fraction yielded in addition to hetisine, alkaloids designated as *hetidine* and *hetisinone*. The latter had been encountered previously as a chemical transformation product of hetisine.

THE PLANT *Aconitum heterophyllum* Wall. (Atis, Ativisha, Ataicha) grows in the Himalaya at altitudes of from 6000 to 15000 feet. The powdered root has been long used in native Indian medicine as a febrifuge and bitter tonic, especially in combating debility after malaria and other fevers.[1] It is also considered efficacious in the treatment of diarrhea, dysentery, cough, dyspepsia and chronic enteritis. Whether the reputed pharmacological properties are due to the alkaloid content is not known. The rhizomes of the plant have been known for many years to contain atisine (I)[2–6] which heads the category of the simpler *Aconitum* alkaloids of low toxicity. Work by Jacobs and Craig[7,8] led to the isolation of three other alkaloids, hetisine (II), heteratisine (III) and benzoylheteratisine (IV). We have carefully re-examined the alkaloid extract from *A. heterophyllum* with the object of isolating new and structurally interesting compounds. This paper reports a study of the total basic extract leading to the isolation of seven new alkaloids.

A 68-kg batch of finely-powdered *A. heterophyllum* roots was thoroughly extracted with 70 per cent SDA-3 solvent (ethanol denatured with 5 per cent methanol) mixture. The extract was concentrated, acidified and extracted with benzene to remove non-basic constituents. The aqueous phase was cooled and progressively basified and extracted with appropriate solvents to give a "weak-base" fraction of 35 g, a "strong-base" fraction of 312 g, and a "very strong-base" fraction of 40 g. The total yield of mixed alkaloids amounted to 387 g (0·57 per cent). In similar extractions of other samples of *A. heterophyllum* roots the amount of basic material ranged from 0·52–0·58 per cent.

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[1] R. N. CHOPRA, I. C. CHOPRA, K. L. HANDA and L. D. KAPUR, *Chopra's Indigenous Drugs of India*, pp. 54–57, U. N. Dhur and Sons Private Ltd., Calcutta (1958).
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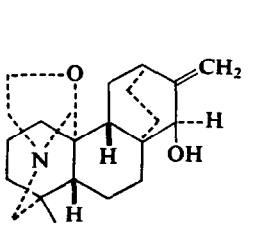
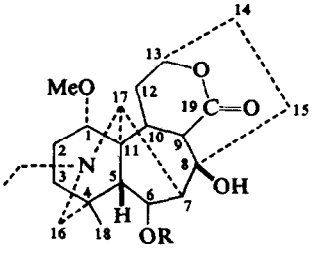
*Alkaloids of the "Strong-Base" Fraction*

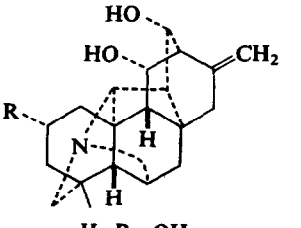
The strong-base fraction containing the bulk of the alkaloids was shown to consist mainly of atisine (I). Since our previous experience had shown that the hydrochlorides of hetisine and heteratisine accompany atisine hydrochloride to a certain extent, fractionation of the strong-base fraction was effected by chromatography over 2400 g of alumina (activity-3). Elution with benzene and benzene–methanol gave 213·8 g of crude atisine (0·31 per cent). Treatment of the latter with hydrochloric acid and crystallization afforded 206 g of pure atisinium



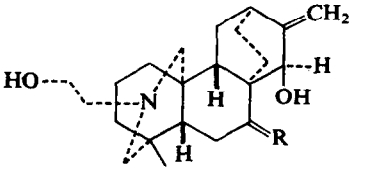
I



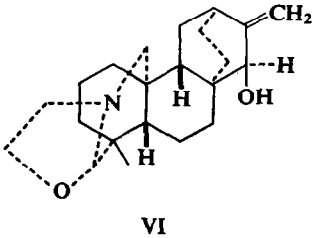
III, R=H
IV, R=Bz



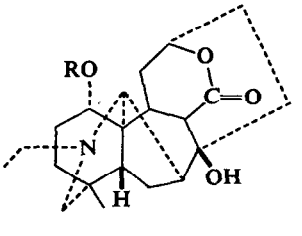
II, R=OH
XI, R=O



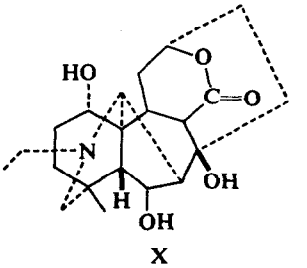
V, R=O
VII, R=$H_2$



VI



VIII, R=$CH_3$
IX, R=H



X

chloride. (See Table 1 for physical constants). Continued elution gave a 28-g fraction containing a new alkaloid, designated *atidine*, (V) $C_{22}H_{33}NO_3$, which crystallized from ether or benzene as truncated prisms, 2·98 g m.p. 182·5–183·5°. Atidine was characterized as the hydrochloride, m.p. 204–125°, and the diacetate hydrochloride, m.p. 182–190°. The structure of this alkaloid has been considered in detail in a recent paper.[9] Continued elution of the column afforded a fraction of 6·2 g of mixed alkaloids. This fraction was combined with mother liquors (22·6 g) from the atidine fraction and rechromatographed. Early fractions from this chromatogram were combined and processed to give 2·5 g of isoatisine (VI), 824 mg of atidine and a small amount of atisine. The total yield of atidine amounted to 3·80 g or

[9] S. W. PELLETIER, *J. Am. Chem. Soc.* **87**, 799 (1965).

0·0056 per cent. Since atisine rapidly isomerizes to isoatisine in alcohol even at room temperature,[10,11] the latter base must be regarded as an artifact.

It was found convenient at this point to monitor the chromatography column by thin layer chromatography on alumina using the system benzene-pyridine-methyl ethyl ketone (20:1:3). Thin layer chromatography showed that a subsequent fraction consisted of isoatisine and an unidentified alkaloid. Separation in chloroform on alumina gave 336 mg of an alkaloid, m.p. 159–161°. This material proved to be F-dihydroatisine (VII),[12,13] as shown by its identity (m.p., TLC, i.r. spectrum) with an authentic sample prepared by the sodium borohydride reduction of atisine. This is the first time that this compound has been reported as a component of *A. heterophyllum*.

Continued elution of the first chromatography column with benzene-methanol (20–100 per cent) gave fractions from which 6·40 g of heteratisine (III),[7,14,15] m.p. 263–267°, was obtained.

*Alkaloids of the "Weak-Base" Fraction*

This fraction (35 g) was separated into lactonic and non-lactonic fractions. The lactone fraction on crystallization from ethanol afforded 12·2 g of heteratisine (III),[7,14,15] m.p. 257–265°. Workup of the mother liquors gave another 1·86 g of heteratisine. Thus the total yield of heteratisine from the strong and weak base fractions is 20·5 g or 0·03 per cent.

Examination of the heteratisine mother liquors accumulated during the processing of 225 kg of *A. heterophyllum* roots revealed the presence of three other lactone alkaloids. These closely related compounds were separated by repeated chromatography over alumina and fractional crystallization to give 25 mg ($1{\cdot}1 \times 10^{-5}$ per cent) of *heterophyllisine* (VIII), $C_{22}H_{33}NO_4$, as fine needles, m.p. 178–179°, $\nu_{max}$ (Nujol) 3584 $cm^{-1}$ (OH), 1727 $cm^{-1}$ (δ-lactone); 113 mg ($5 \times 10^{-5}$ per cent) of *heterophylline* (IX), $C_{21}H_{31}NO_4$, as prisms, m.p. 221·5–223°, 3534 $cm^{-1}$ (OH), 1748 $cm^{-1}$ (δ-lactone) and 112 mg ($5 \times 10^{-5}$ per cent) of *heterophyllidine* (X), $C_{21}H_{31}NO_5$, as heavy prisms, m.p. 269–272°, $\nu_{max}$ 3546, 3205, 2778 $cm^{-1}$ (OH), 1748 $cm^{-1}$ (δ-lactone). The structures indicated for these three new alkaloids are based on NMR and mass spectral data.[16]

*Alkaloids of the "Very Strong Base" Fraction*

This fraction (40 g) yielded 30·59 g of hetisine hydrochloride,[7] representing 27·6 g of hetisine (II) (0·046 per cent). In another experiment the mother liquors resulting after removal of hetisine from 17·8 g of "Very Strong Base" fraction were freed of possible contamination with atisine by refluxing in ethanol for 90 min to convert any atisine to isoatisine, converting the mixture to the sulfates and removal of the isoatisine at pH 10 by extraction with benzene. The aqueous solution yielded a crude basic fraction which was partitioned at pH 10 between water and chloroform. The chloroform extract yielded 3·9 g which was separated by repeated chromatography and crystallization to give 116 mg of a new alkaloid named *hetidine*, $C_{21}H_{27}NO_4$, m.p. 217–220° and 62 mg of a compound designated as *hetisinone* (dehydrohetisine) (XI), m.p. 273–275°, and which has been prepared previously by oxidation of

[10] S. W. Pelletier, K. W. Gopinath and K. Kawazu, *Chem. & Ind.* **28** (1966).
[11] S. W. Pelletier, *Experientia* **20**, 1 (1964).
[12] W. A. Jacobs and L. C. Craig, *J. Biol. Chem.* **147**, 567 (1943).
[13] O. E. Edwards and T. Singh, *Can. J. Chem.* **32**, 465 (1954).
[14] M. Przybylska, *Can. J. Chem.* **41**, 2911 (1963).
[15] R. Aneja and S. W. Pelletier, *Tetrahedron Letters* **12**, 669 (1964).
[16] S. W. Pelletier and R. Aneja, *Tetrahedron Letters* **6**, 557 (1967).

hetisine diacetate followed by saponification.[17] What appears to be the same compound (dehydrohetisine) has recently been isolated from *Delphinium Cardinale* Hook.[18] That hetisinone is not an artifact formed from hetisine during processing of the mother liquors was demonstrated by treating hetisine with 50 per cent sodium hydroxide for 48 hr, and allowing hetisine to stand on an alumina column for 48 hr. Hetisine was recovered unchanged from both conditions.

Analytical and spectroscopic data on hetidine suggest that the compound may have a structure in which the nitrogen is not involved in an heterocyclic ring. The NMR spectrum shows a 3H singlet at $\tau$ 8·83 for a $CCH_3$, a 6H singlet at $\tau$ 7·54 for $-N(CH_3)_2$ and a 2H doublet at $\tau$ 5·02 and 5·20 for the exocyclic methylene group. Multiple carbonyl groups are indicated by infrared absorption at 1710 and 1680 $cm^{-1}$ and hydroxyl absorption at 3400 $cm^{-1}$. Structural work continues on hetidine and will be reported at a later date.

TABLE 1.

| Name | m.p. (cor.) | $[\alpha]_D$ | pKa' (50% MeOH) |
|---|---|---|---|
| Atisine | resin | −30·7° (EtOH) | 12·8 |
| Atisinium Chloride | 329–331° dec. | +26·6° (EtOH) | — |
| Atidine | 182·5–183·5° | −47·0 (Chf.) | 7·53 |
| Atidine·HCl | 204–215° | — | — |
| Atidine diacetate·HCl | 182–190° | — | — |
| F-Dihydroatisine | 159–161° | −44·5° (EtOH) | 8·31 |
| F-Dihydroatisine·HCl | 260–269° dec. | — | — |
| Heteratisine | 267–269° | +26·0° (Chf.) | 8·77 |
| Heterophyllisine | 178–179° | +15·5° (MeOH) | — |
| Heterophylline | 221·5–223° | +10·5° (MeOH) | — |
| Heterophyllidine | 269–272° | +42·3° (MeOH) | — |
| Hetisine | 256·5–259° | +10·9° (Chf.) | 10·0 |
| Hetidine | 218–221° | — | — |
| Hetisinone (dehydrohetisine) | 275–278° | — | — |
| Isoatisine | 149·5–152° | −22·4° (EtOH) | 10·3 |
| Isoatisine·HCl | 312–315° | −6·9° (EtOH) | — |

## EXPERIMENTAL

### *General Experimental Procedures*

Melting points are corrected and were taken on a hot-stage equipped with a microscope and polarizer. Finely powdered samples were placed on the stage 15° below the m.p. and the temperature raised at a rate of about 4°/min. Rotations were taken in $CHCl_3$ unless otherwise noted. Ultraviolet spectra were determined in 95 per cent ethanol on a Beckman model DU spectrophotometer or a Perkin Elmer Model 202 spectrometer, and i.r. spectra on Perkin Elmer Model 21 and Infracord spectrophotometers. Nuclear magnetic resonance spectra were taken on a Varian A-60 spectrometer in deuterochloroform with tetramethylsilane as an internal standard. Light petroleum refers to a fraction b.p. 30–70°. Ligroin refers to a fraction b.p. 60–70°. The removal of solvents *in vacuo* was accomplished with a Craig-type rotating flash evaporator at 15–20 mm and with the water bath usually at 35–50°.

### *Processing of Aconitum Heterophyllum Roots*

The dry, powdered roots (68 kg) were percolated three times at room temperature with 70 per cent SDA-3 (ethanol denatured with 5 per cent methanol), using 4 l. of solvent per kilogram for each percolation. The roots were squeezed dry after each percolation. The extract was concentrated *in vacuo* below 35° to 8 l.

The thick concentrate was divided into roughly eight equal portions and each portion processed separately. Then similar portions from the separation scheme were combined. 1 l. of the extract was dissolved in 2 l. of

[17] S. W. PELLETIER and A. J. SOLO, unpublished work.
[18] M. H. BENN, *Can. J. Chem.* **44**, 1 (1966).

1½ per cent $H_2SO_4$, treated with 10 g of filter cel and filtered. The filtrate (3 l.) was extracted eight times with 1-l. portions of benzene. The benzene extract containing neutral components was reserved as fraction (N). The aqueous phase was stored overnight at 5°, neutralized with cold 20 per cent NaOH to pH 7 (about 190 ml required) and then with 10 per cent $Na_2CO_3$ to pH 8·2 (75–100 ml required). Extraction with five 1-l. portions of benzene gave a weak base fraction designated WB. The aqueous phase was again stored overnight at 5°, covered with 1 l. of benzene and treated with 150 ml of cold 50 per cent NaOH (pH > 12). Extraction with five 1-l. portions of benzene gave a strong base fraction designated (B). The cold aqueous phase was treated with 150 ml of 50 per cent NaOH and rapidly extracted with five 1-l. portions of $CHCl_3$. This fraction containing most of the hetisine was designated (C). Continuous extraction of the aqueous solution with chloroform for 6 days yielded negligible material.

*Strong Base Fraction (B)*

These fractions were combined, treated with 30 g of $Na_2SO_4$ and filtered. An aliquot showed the fraction amounted to 312 g. Since previous experience demonstrated that the HCl-ides of hetisine and heteratisine accompany atisinium chloride to a certain extent during crystallization, it was necessary to carry out a preliminary chromatographic separation. The mixture was chromatographed in benzene over 2400 g of Merck alumina (activity 3) as shown in the table below.

TABLE 2.

| Fraction | Solvent | Liters | Weight |
|---|---|---|---|
| 1 | Benzene | 1·0 | 22·10 g |
| 2–3 | Benzene | 1·0 | 146·40 |
| 4–6 | Benzene | 2·0 | 16·40 |
| 7–9 | Benzene-1% MeOH | 3·0 | 6·50 |
| 10–11 | Benzene-1% MeOH | 2·0 | 4·50 |
| 12–13 | Benzene-2% MeOH | 2·0 | 17·95 |
| 14–17 | Benzene-2% MeOH | 2·5 | 28·35 |
| 18–21 | Benzene-2% MeOH | 5·0 | 5·05 |
| 22–23 | Benzene-10% MeOH | 2·0 | 1·20 |
| 24–27 | Benzene-10% MeOH | 4·0 | 13·00 |
| 28–30 | Benzene-10% MeOH | 3·5 | 2·05 |
| 31–35 | Benzene-20% MeOH | 5·0 | 1·40 |
| 36–40 | MeOH | 5·0 | 1·30 |
| 41–52 | MeOH-5% AcOH | 18·0 | 40·95 |
| | | Total | 307·15 g |

*Fractions* 1–6, *Atisine*. (184·9 g)

Material from these fractions in 500 ml of cold absolute ethanol was treated with a slight excess of cold conc. HCl. Atisinium chloride crystallized as a mush of fine needles, 162 g, m.p. 312–315° dec., $[\alpha]_D^{32}+19·6$ (c 1·45 in 95 per cent EtOH). Concentration of the mother liquors afforded another 23·3 g of less pure atisinium chloride, m.p. 308–312° dec. An analytical sample showed m.p. 329–331° dec., $[\alpha]_D^{30}+26·6$, 23·3 (c 1·0 in 95 per cent EtOH); $\nu_{max}$ (KBr) 3300, 3226 $cm^{-1}$ (OH), 1680 $cm^{-1}$ ($\rangle\overset{+}{N}{=}C$), 3040, 1654, 891 $cm^{-1}$ ($\rangle C{=}CH_2$). (Calcd. for $C_{22}H_{33}NO_2 \cdot HCl$: C, 69·52; H, 9·02. Found: C, 69·79; H, 8·94 per cent).

The free base was liberated from an ice-cold aqueous solution of the chloride with dil. NaOH and extracted quickly with benzene. Evaporation gave atisine as a colorless resin or foam $[\alpha]_D-30·7°$. Infrared spectrum with $CaF_2$ prism: $\nu_{max}$ (Nujol): 3390 $cm^{-1}$ broad (OH), 3067, 1647 ($\rangle C{=}CH_2$); NaCl prism: $\nu_{max}$ (film from chloroform) 3413 $cm^{-1}$ broad (OH), 3063, 1650, 894 $cm^{-1}$ ($\rangle C{=}CH_2$), 1370 $cm^{-1}$ ($CCH_3$). Atisine in 50 per cent methanol (0·0098 N) showed a pKa′ of 12·8. NaOH at the same concentration gave a pH of 12·9.

*Fractions* 7–13. (28·95 g)

Treatment of this material in absolute ethanol with conc. HCl gave 20·69 g of atisinium chloride.

*Fractions* 14–17, *Atidine*

Material from these fractions (28·35 g) crystallized from ether as truncated prisms, 2·23 g, m.p. 176–181°. Recrystallization from ether or benzene gave pure atidine, m.p. 182·5–183·5°, $[\alpha]_D^{31}-47$ (c 1·7 in chf.). The

equivalent weight was determined by electrometric titration with 0·0447 N HCl in 50 per cent methanol. Calcd. 359·5, Found: 356·3, pKa' 7·53; $\nu_{max}$ (KBr) 3544, 3454 $cm^{-1}$ (OH), 1695 $cm^{-1}$ (>C=O), 3086, 1658, 900 $cm^{-1}$ (>C=$CH_2$), 1376 $cm^{-1}$ (C-$CH_3$). (Calcd. for $C_{22}H_{33}NO_3$: C, 73·50; H, 9·25; N, 3·90. Found: C, 73·57, 73·74; H, 9·42, 9·39; N, 3·93 per cent.)

Concentration of the mother liquors and rechromatography in benzene over 175 g of Merck alumina gave several fractions which crystallized from benzene to give 752 mg of atidine, m.p. 182–184°, $[\alpha]_D^{29} -47°$ (c 1·8 in chf.).

*Fractions* (18–23), *Atisine, Isoatisine, Atidine, F-dihydroatisine*

These fractions (6·25 g) were combined with the mother liquors remaining after the isolation of atidine from fractions (14–17) (total 22·6 g) and chromatographed in benzene over 150 g of alumina. The first five fractions consisting of 11 g were treated with conc. HCl in ethanol. A crystalline fraction separated, m.p. 289–293° dec. (4·3 g). The non-crystalline residue was reserved and is described subsequently. The crystalline HCl-ide was dissolved in water, cooled and treated with $Na_2CO_3$. Extraction of the solution gave material which on fractional crystallization afforded 1·20 g of isoatisine, m.p. 149·5–155° cor., the i.r. spectrum of which was identical with that of an authentic sample. Extraction of the solution after addition of NaOH gave 485 mg of resin which consisted mainly of atisine. Boiling the atisine resin in ethanol for 2 hr isomerized it to isoatisine, m.p. 142–145°.

The non-crystalline fraction of the above HCl-ide (7·12 g) was converted to its base and chromatographed over 200 g of Woelm neutral alumina (activity grade 3). Elution with benzene gave fractions (1–9)A which consisted of crude isoatisine (1·3 g). Fractional crystallization from acetone gave 0·34 g of isoatisine, m.p. 145–151°.

Fractions (10–12)A (733 mg) were eluted with ether and crystallized from acetone to give atidine, m.p. 181–183°.

Fractions (13–19)A were eluted with ether and ether–methanol (3 per cent) to give 600 mg. This material did not crystallize and was not studied further.

Fractions (20–33)A were eluted with ether–methanol (10 per cent) to give 1·3 g of resin which did not crystallize and was not studied further.

The mother liquors from fractions (1–9)A were combined (3·5 g) and rechromatographed in benzene over 120 g of Woelm neutral alumina. The column was monitored by TLC on alumina using the system benzene-pyridine-methyl ethyl ketone (20:1:3). Fractions (1–8)B amounted to 2·11 g and consisted of isoatisine and an unknown component. Thin layer chromatography showed that this component could be separated from isoatisine by chromatography in chloroform. Separation gave 336 mg, m.p. 159–161°, which was shown to be *F-dihydroatisine*[12, 13] by m.p. and i.r. spectrum in Nujol.

Fractions (9–10)B, (250 mg), by TLC showed the presence of iosatisine, traces of atidine and F-dihydroatisine, and a small amount of an unknown component.

Fractions (11–13)B (307 mg) on recrystallization from acetone afforded 91 mg of atidine, m.p. 180–182°.

Fractions (14–27)B (670 mg) by TLC showed the presence of isoatisine, atidine and traces of several other components.

*Fractions* 24–27, *Heteratisine*

These fractions (13·0 g) were evaporated to dryness, taken up in ethanol and allowed to stand for a year. Huge prisms of *heteratisine* separated, 4·0 g, m.p. 261–270°. The mother liquors were processed to recover any additional heteratisine as follows. The material was boiled in methanolic KOH for 30 min, concentrated to remove methanol and taken up in water. Extraction with $CHCl_3$ removed non-heteratisine containing bases. The aqueous solution containing any heteratisine as a carboxylate salt was made acid to Congo Red with $H_2SO_4$, warmed briefly on the steam bath to effect lactonization and then made ice-cold. The solution was neutralized to pH 7 with cold, dil NaOH and then brought to pH 8·5–9·0 with cold $Na_2CO_3$ solution. The cold mixture was rapidly extracted with $CHCl_3$. The extract yielded a resin which crystallized from benzene to give 700 mg of prisms with an i.r. spectrum identical with that of heteratisine.

*Fractions* 28–30. (2·05 g)

This material was dissolved in 2 per cent $H_2SO_4$ and extracted with benzene to remove occluded neutral material. The dark acid extract was treated with Norit, filtered, chilled with crushed ice and brought to pH 8·5 with $Na_2CO_3$. Extraction with $CHCl_3$ gave a resin which crystallized from methanol to give 1·26 g of prisms, m.p. 262–271°, with an i.r. spectrum identical with that of heteratisine.

*Fractions* 31–40. (2·70 g)

These very dark fractions were partitioned between 2 per cent $H_2SO_4$ and $CHCl_3$. The acidic extracts were treated with Norit, filtered and while cold, extracted repeatedly with $CHCl_3$ as the solution was made progressively more basic with $NaHCO_3$, $Na_2CO_3$ and NaOH. Since tests showed that the various extracts con-

tained heteratisine, they were combined and processed together. The combined extracts (1·15 g) in methanol were boiled for 30 min with KOH, diluted and extracted with $CHCl_3$. The $CHCl_3$ removed 534 mg which was discarded. The aqueous phase was made acid to Congo Red with $H_2SO_4$, warmed briefly to effect lactonization, and then clarified with Norit. After the clear yellow filtrate was brought to 5° with crushed ice, the pH was adjusted to pH 7 with $NaHCO_3$ and then to 8·5 with carbonate. Extraction with $CHCl_3$ gave 421 mg of a resin which crystallized as characteristic prisms, 385 mg, m.p. 260–265°, with an i.r. spectrum identical with that of heteratisine.

*Fractions* 41–52. (40·95 g)

Since most of these fractions contained Al- salts, they were combined in water, chilled, made strongly basic with NaOH and extracted several times with $CHCl_3$. Extraction gave 2·78 g of black tar. This was again partitioned between $CHCl_3$ and 5 per cent $H_2SO_4$. The acid extract was made strongly basic with NaOH and heated for 30 min to saponify any heteratisine present. The basic mixture was extracted with $CHCl_3$ to give a dark resin which was discarded. The aqueous solution was made acid to Congo, warmed briefly to lactonize the opened lactone, and then brought to pH 8·5 with $Na_2CO_3$ at ice bath temperature. Extraction with $CHCl_3$ gave 110 mg of dark resin which crystallized from methanol as large prisms of heteratisine, 63 mg. The identity was confirmed by the i.r. spectrum.

"*Weak Base*" *Fraction. (WB)*

The total weak base fraction was dissolved in 1 l. of hot benzene. On cooling some insoluble material separated. The benzene solution was extracted eleven times with 200-ml portions of 2 per cent $H_2SO_4$. During extraction a small amount of an insoluble sulfate separated on the walls of the funnel and was reserved. The combined acidic extracts were filtered through Celite, crushed ice added and the pH adjusted to 9·0 by addition of $Na_2CO_3$ solution. Extraction with $CHCl_3$ gave 35·0 g of bases. Heteratisine was separated from other non-lactonic components by the following procedure. A solution of the 35 g of bases in 300 ml of 80 per cent methanol was treated with 30 g of KOH and boiled for 20 min. After removal of methanol, the solution was diluted with one l. of water and extracted with $CHCl_3$. Evaporation of the solvent gave 11·6 g of non-lactonic bases. The aqueous phase containing any heteratisine as a carboxylate salt was made acid to Congo Red with $H_2SO_4$, warmed on the steam bath for a few minutes to effect lactonization, treated with Norit and filtered. After cooling the clear yellow solution to 0° with crushed ice, the pH was adjusted to 9·0 with cold $Na_2CO_3$ solution and the solution extracted 15 times with $CHCl_3$. Evaporation yielded 15·23 g of a semicrystalline residue. Crystallization from ethanol gave crops of crystals melting between 257–265° dec. and totaling 12·26 g. Recrystallization from ethanol gave 10·0 g of heteratisine, m.p. 261–265·5° dec. and 1·81 g, m.p. 254–262° dec. An analytical sample melted at 263–267°, with the melting rather dependent on the rate of heating and the temperature at which the sample was placed on the block. $\nu_{max}$ (Nujol, $CaF_2$ prism) 3460, 3401 $cm^{-1}$ (OH), 1738 $cm^{-1}$ (δ-lactone); $\nu_{max}$ (Chf, $CaF_2$ prism): 3378, 1724 $cm^{-1}$; $\nu_{max}$ (KBr, NaCl prism): 1382 $cm^{-1}$ (C–$CH_3$). (Calcd. for $C_{22}H_{33}NO_5$: C, 67·49; H, 8·50; N, 3·58; $OCH_3$, 7·93; C–$CH_3$, 3·85. Found: C, 67·57, 67·55, 67·57; H, 8·60, 8·53, 8·31; N, 3·74, 3·69; $OCH_3$, 7·62; C–$CH_3$, 3·64, 3·71 per cent.)

*Isolation of Heterophyllisine, Heterophylline and Heterophyllidine*

The mother liquors remaining from heteratisine fractions obtained from about 225 kg of *Aconitum heterophyllum* roots were combined, dissolved in methanol containing 5 g of KOH and refluxed for 15 min. The mixture was evaporated to dryness *in vacuo*, taken up in water and extracted with $CHCl_3$ to remove traces of non-lactonic components. The brown colored aqueous solution was made acid to Congo Red with dil. $H_2SO_4$, warmed briefly to effect lactonization, treated with Norit and filtered. The pale yellow filtrate was chilled to 5°, made basic to pH 9 and quickly extracted with cold $CHCl_3$. Evaporation gave 2·47 g of brown resin. Thin layer chromatography over alumina using four different solvent systems showed this resin contained at least three components. It was chromatographed over 125 g of neutral Woelm alumina (activity 3).

TABLE 3.

| Fraction | Solvent | ml | Weight, mg |
|---|---|---|---|
| 1, 2 | benzene | 150 | 17·9 |
| 3–9 | Benzene-acetone (19:1) | 550 | 199·0 |
| 10–19 | Benzene-acetone (9:1) | 2125 | 552·8 |
| 20–23 | Acetone | 900 | 195·0 |
| 24–29 | Benzene-methanol (9:1) | 1800 | 1282·5 |
| 30–31 | Methanol | 750 | 73·5 |
| | | Total | 2320·7 mg |

*Heterophylline*

Thin layer chromatography (benzene–acetone, 1:1) showed that fractions (3–9) contained two components. This material was combined and processed to yield heterophyllisine (see below). Fractions (10–19) crystallized from acetone with melting points varying from 204–219°. These fractions were combined (552 mg) and crystallized thrice from acetone to give 169 mg of leaflets, m.p. 219·0–222·5°. Repeated crystallization of material from the mother liquors gave 54 mg of 216–219°. This was combined with the 169-mg. sample and recrystallized twice from methanol to give 113 mg of prisms of *heterophylline*, m.p. 221·5–223°, $[\alpha]_D^{30}+10·5$ (c, 2·0 MeOH); $\nu_{max}$ (Nujol) 3534 $cm^{-1}$, sharp (OH), 1748 $cm^{-1}$ (δ-lactone); $\tau$ 9·08. (3H singlet, C–C$H_3$), $\tau$ 8·87 (3H, $J$=7·5 c/s, –NC$H_2$C$H_3$), $\tau$ 5·14 (1H mult., $H$–C(13)–OCO–), $\tau$ 6·70 (1 H-width at ½ ht.=3 c/s, H–C(17)N–), $\tau$ 6·18 (1H doublet, $J$=4 c/s, $H$C(9)CO–O–); no methoxyl resonance. (Calcd. for $C_{21}H_{31}NO_4$; C, 69·78; H, 8·64, N, 3·87. Found: C, 70·03, 70·00; H, 8·58, 8·57; N, 4·22; $OCH_3$, 0·19 per cent); M.W. calc. 361·47.; mass peak m/e=361.

An X-ray study showed unit cell dimensions of 15·78 A°, 12·14 A°, 9·596 A° with space group $P2_12_12_1$; $Z$=4, crystal density = 1·3109 g/$cm^3$ at 25°. The molecular weight calculated from the X-ray data is 363·25 ± 2.

*Heterophylline acetate*

A solution of 23 mg. of heterophylline in a mixture of 0·1 ml of pyridine and 0·1 ml of acetic anhydride was allowed to stand at room temperature for 18 hr. Evaporation to dryness *in vacuo* gave a residue which was taken up in water, treated with $NaHCO_3$ and extracted with benzene. Evaporation gave a residue which crystallized from ether–light petroleum as prismatic needles, m.p. 174–176°. (Calcd. for $C_{23}H_{33}NO_5$: C, 68·46; H, 8·24. Found: C, 68·68; H, 8·33 per cent).

*Heterophyllidine*

Fraction (20–31) by TLC (benzene–acetone 1:1) showed the presence of heteratisine and another component. Crystallization from methanol afforded a small amount of heteratisine. The mother liquors were combined with those from fractions (10–19) to give 1·32 g. This was rechromatographed in benzene over 40 g of alumina.

TABLE 4.

| Fraction (B) | Solvent | ml | Weight, mg |
|---|---|---|---|
| 1–5 | Benzene | 1000 | 149 |
| 6–8 | Benzene–MeOH (99:1) | 150 | 459 |
| 9 | Benzene–MeOH (99:1) | 50 | 83 |
| 10–13 | Benzene–MeOH (99:1) | 850 | 339 |
| 14–15 | Benzene–MeOH (9:1) | 500 | 157 |
| 16 | Benzene–MeOH (4:1) | 375 | 13·5 |

Fractions B (1–5) were combined with (3–9) and reserved.

Fractions B (6–8) crystallized from acetone to give some heteratisine. The mother liquors (200 mg) were added to fractions (3–9) and B (1–5).

Fractions B (10–15) were combined and crystallized twice from methanol to give 112 mg of heavy prisms of *heterophyllidine*, m.p. 269–72°; $[\alpha]_D^{26°}+43·3°$ (c, 1·26 in methanol) $\nu_{max}$ 3546, 3205, 2778 $cm^{-1}$ (OH), 1748 $cm^{-1}$ (δ-lactone); $\tau$ 8·92 [3H singlet, C–C$H_3$ superimposed on 3H triplet, $J$=6·5 c/s, NC$H_2$C$H_3$], $\tau$ 6·57 [1H doublet, $J$=1·5 c/s, $H$–C(17)N–], $\tau$ 6·19 [1H, unresolved, $H$–C(9)CO–O–], $\tau$ 5·33 [1H mult. $H$–C(6)O–], $\tau$ 5·08 [1H, mult. $H$C(13)OCO–]. No methoxyl absorption. (Calcd. for $C_{21}H_{31}NO_5$: C, 66·82; H, 8·28. Found: C, 66·74; H, 8·51 per cent); M.W. Calc. 377·47. M.W. 377 (mass peak).

*Fraction* (3–9), *B*(1–5) *and B*(6–8) *ML., Heterophyllisine*

These fractions (532 mg) were chromatographed in benzene over 15 g of Woelm neutral alumina.

TABLE 5.

| Fraction | Solvent | ml | Weight, mg |
|---|---|---|---|
| 1–3 | Benzene | 150 | 150 |
| 4–6 | Benzene | 900 | 92 |
| 7 | Benzene | 300 | 2 |
| 8 | Ether | 500 | 8 |
| 9–14 | Benzene-MeOH (199:1) | 1200 | 120 |
| 15–16 | Benzene-MeOH (99:1) | 1070 | 101 |

Fractions (1–6) were combined and crystallized from acetone to give 25 mg of fine needles of heterophyllisine, m.p. 178–179°; $[\alpha]_D^{27}+15{\cdot}5°$ (c 0·90, methanol). Heterophyllisine was homogeneous by TLC on alumina (benzene–acetone 1:1, $R_f$ 0·85) and on silica gel (chloroform–methanol 19:1, $R_f$ 0·20). $\nu_{max}$ (Nujol) 3584 $cm^{-1}$ (OH), 1727 $cm^{-1}$ (δ-lactone); $\tau$ 9·19 (3H singlet, $-CCH_3$), $\tau$ 8·94 (3H triplet, $J=7$ c/s, $-NCH_2CH_3$), $\tau$ 7·90 1H singlet, which disappears on addition of $D_2O$, $-OH$); $\tau$ 6·72 (3H singlet, $OCH_3$) $\tau$ 6·62 (1H, doublet, $J=1$ c/s, probably H–C(17)N–), $\tau$ 5·26 (1H, broad multiplet, H–C(13)–OCO–). (Calcd. for $C_{22}H_{33}NO_4$: M.W. 375·49. Found: m/e 375).

*"Very Strong Base Fraction"(C), Hetisine*

A solution of this material (40·0 g) in absolute ethanol was treated with a slight excess of conc. HCl and let stand overnight in the refrigerator. Herisine hydrochloride separated as needles, 30·59 g. The mother liquors were processed as described below.

A solution of 24·6 g of the crude HCl-ide in 500 ml of water was made basic with a large excess of 50 per cent NaOH and extracted with 10 × 150-ml $CHCl_3$. The extracts were combined, washed, filtered through a bed of Filter-cel, concentrated to dryness *in vacuo* and flashed several times with methanol. The residue was crystallized from acetone to give 14·34 g of hetisine, m.p. 253–257°. Concentration of the mother liquors and recrystallization of the resulting crystals gave 4·81 g of hetisine, m.p. 255–259°.

An analytical sample prepared by crystallization from methanol–acetone, melted at 256·5–259°, $[\alpha]_D^{27}+10{\cdot}9°$ (c 1·5 in chf.). Infrared spectrum (Nujol, $CaF_2$ prism): $\nu_{max}$ 1657, 1654, 3067 $cm^{-1}$ (>C=$CH_2$); (Nujol, NaCl prism): $\nu_{max}$ 900, 1661 s, 1650, 3049 $cm^{-1}$ (>C=$CH_2$), 3165, 3311, 3425 (OH); ($CHCl_3$, $CaF_2$ prism): $\nu_{max}$ 1653 $cm^{-1}$ (>C=$CH_2$), 3413 $cm^{-1}$ (envelope, OH); (in KBr, NaCl prism): $\nu_{max}$ 900, 1653, 3030 (C=$CH_2$), 3390 (envelope, OH), 1379 $cm^{-1}$ (C–$CH_3$).

(Calcd. for $C_{20}H_{27}NO_3$: C, 72·92; H, 8·26; N, 4·25; C–$CH_3$, 4·57. Found: C, 72·92, 73·04; H, 8·19, 8·32; N, 4·35; C–$CH_3$, 0·57, 0·61 per cent.)

*Mother Liquors From Hetisine*

In another experiment 17·8 g of fraction "C" in 100 ml of ethanol was refluxed for 90 min to isomerize any contaminating atisine to isoatisine. After standing overnight, solvent was removed *in vacuo*, the residue taken up in 400 ml of 2 per cent $H_2SO_4$, basified to pH 10 with 25 per cent NaOH and extracted with three 300-ml portions of benzene. A small amount of insoluble gum separated at this point. The benzene extract containing isoatisine was reserved.

The aqueous solution along with the insoluble gum was made strongly basic by addition of 100 ml of 50 per cent NaOH and extracted repeatedly with chloroform. Evaporation of the solvent gave 16 g of residue which was taken up in 120 ml of ethanol and made acidic to Congo Red with conc. HCl. After 1·5 hr in the refrigerator, 5·42 g of hetisine hydrochloride was collected.

The mother liquors from the 5·42 g of hetisine hydrochloride were evaporated *in vacuo*, the residue dissolved in water, basified to pH 10 with 25 per cent NaOH and extracted with $CHCl_3$. Evaporation of the solvent gave 3·9 g of residue (CA). When the aqueous solution was basified to >pH 14 with NaOH and extracted with ether, 4·1 g of base was isolated (CB).

*Hetidine and Hetisinone from Fraction (CA)*

In a typical experiment 2·0 g of fraction -CA was chromatographed in chloroform over 40 g of Woelm neutral alumina (activity 5). The first four fractions totaling 100 ml eluted 871 mg of resinous product. This was rechromatographed in benzene over 30 g of Woelm neutral alumina (activity 5) as shown below.

TABLE 6.

| Fraction | Solvent | ml | Weight, mg |
|---|---|---|---|
| 1–6 | Benzene | 150 | 98·4 oil |
| 7–11 | Benzene | 400 | 188·1 solid |
| 12, 13 | Benzene–ether (9:1) | 275 | 21·6 solid |
| 14 | Benzene–MeOH (99:1) | 50 | 42·4 oil |
| 15 | Benzene–MeOH (99:1) | 50 | 54·8 solid |
| 16 | Benzene–MeOH (99:1) | 50 | 62·2 solid |
| 17, 18 | Benzene–MeOH (99:1) | 280 | 21·1 gum |

*Fractions* (7–13). *Hetidine*

These fractions were combined (209·7 mg) and crystallized from benzene–hexane to give 126 mg, m.p. 191–197°. Recrystallization from the same solvent gave 116 mg, m.p. 217–220°. Further crystallization gave 93·4 mg of essentially the same melting point (218–221°); $\nu_{max}$ (Nujol) 3400 $cm^{-1}$ (broad, –OH), 1710, 1680 $cm^{-1}$ (carbonyls), 905 $cm^{-1}$ (>C=$CH_2$); $\lambda_{max}$ (EtOH) 209 mμ (=5200); τ 8·83 (3H singlet, CC$H_3$), τ 7·54 (6H singlet, –N($CH_3$)$_2$), τ 5·02 and 5·20 (2H, >C=$CH_2$), τ 2·65 (benzene solvate). The benzene present as solvate was not removed by drying in high vacuum. Calcd. for $C_{21}H_{27}NO_4$: C, 70·56; H, 7·61.

Found: C, 70·9, 71·02; H, 7·72, 7·70 per cent.

Crystallization of a sample of hetidine from acetone gave needles, m.p. 219–220° the NMR spectra of which no longer showed benzene absorption at τ 2·65. The mass spectrum of a sample gave m/e of 357 (calc. 357·5).

*Fractions* (15–16). *Hetisinone*

These fractions were combined (76·8 mg and crystallized from benzene to give 62 mg of rhombs of m.p. 273–275°; $\nu_{max}$ (Nujol) 3570 $cm^{-1}$ (OH), 1710 $cm^{-1}$ (>C=O), 1650, 890 $cm^{-1}$ (>C=$CH_2$); τ 8·85 (3H singlet, –CC$H_3$), τ 5·12 and 5·25 (2H, >C=$CH_2$). The i.r. and NMR spectra were identical with that of an authentic sample of hetisinone.[17]

*F-Dihydroatisine.—A. From Atisine Hydrochloride*

A solution of 2·0 g of atisinium chloride in 50 ml of 80 per cent methanol was treated with 1·4 g of $NaBH_4$. After standing 2 hr the solution was evaporated to dryness *in vacuo*. The residue was taken up in water and extracted several times with $CHCl_3$. Crystallization of the extract from aqueous methanol gave flat blades, m.p. 158–159·5°, $[\alpha]_D^{31}$ −44·5° (c, 2·21 in EtOH); $\nu_{max}$ (Nujol), 3425, 3356 Sh. $cm^{-1}$ (OH), 3058, 1650, 889 $cm^{-1}$ (>C=$CH_2$), $\nu_{max}$ (KBr) 3413 (OH), 3058, 1653, 885 $cm^{-1}$ (>C=$CH_2$), 1370 $cm^{-1}$ (C—$CH_3$), τ 9·22 (1H singlet, CC$H_3$), τ 6·34, 2H triplet, $J$=6 c/s, (–$NCH_2CH_2OH$), τ 4·92, 2H quartet, $J$=2 c/s (>C=$CH_2$). (Calcd. for $C_{22}H_{35}NO_2$: C, 76·47; H, 10·21; C–$CH_3$, 4·36. Found: C, 76·80, 76·34; H, 10·12, 10·10; C–$CH_3$, 4·19 per cent.)

*B. From Isoatisine*

Reduction of isoatisine in 90 per cent methanol with $NaBH_4$ gave dihydroatisine in essentially quantitative yields, m.p. 158–159°.

*F-Dihydroatisine Hydrochloride*

Treatment of 101 mg of dihydroatisine in 10 ml of acetone with a slight excess of conc. HCl caused an immediate separation of fine needles of the hydrochloride, 107 mg, m.p. 260–269° dec. $\nu_{max}$ (Nujol) 3400, 3300 $cm^{-1}$ (OH), 2717, 1798 $cm^{-1}$ ($\overset{+}{N}H$), 1656, 892 $cm^{-1}$ (>C=$CH_2$). (Calcd. for $C_{22}H_{35}NO_2 \cdot HCl$: C, 69·17; H, 9·50. Found: C, 69·23; H, 9·31 per cent.)

*Isomerization of Atisine to Isoatisine. A. With Alkali*

A solution of 23·2 g of atisinium chloride, m.p. 312–315°, in 350 ml of cold water was treated with an excess of 10 per cent NaOH and quickly extracted with benzene. Evaporation of the solvent gave 20 g of atisine resin. The latter was dissolved in 200 ml of methanol, treated with a solution of 10 g of KOH in 20 ml of water and boiled under reflux for 10 min. The mixture was concentrated *in vacuo*, diluted with water and extracted with $CHCl_3$. The extract yielded material which crystallized from acetone to give 12·4 g of isoatisine, m.p. 143–151°. Concentration of the mother liquor afforded an additional 2·15 g of isoatisine. The syrup remaining did not crystallize readily and apparently consisted of some unchanged atisine. It was dissolved in methanol, treated with 3·0 g of KOH and boiled under reflux for 1 hr. Extraction with $CHCl_3$ gave material which crystallized from acetone to give 3·52 g of isoatisine. An analytical sample melted at 148–153°, $[\alpha]_D^{33}$ −22·4° (c 1·45 in 95 per cent EtOH); $\nu_{max}$ (Nujol) 3385 $cm^{-1}$ (OH), 1653, 894 $cm^{-1}$ (>C=$CH_2$); $\nu_{max}$ (KBr) 3356 $cm^{-1}$ (OH), 3012, 1656, 893 $cm^{-1}$ (>C=$CH_2$); 1385 $cm^{-1}$ (C–$CH_3$).

*Isoatisinium chloride*

The salt was prepared by treating isoatisine with conc. HCl in cold acetone. Crystallization from acetone gave needles, m.p. 304–307°, $[\alpha]_D^{30}$ −6·9° (c 1·17 in 95 per cent EtOH).

*In Boiling Methanol*

A solution of 317 mg of atisine in 15 ml of methanol was boiled under reflux for 24 hr. Evaporation and crystallization from acetone gave 201 mg of isoatisine, m.p. 147–150·5°, identical in all respects with a sample of isoatisine prepared by process (A). Concentration of the mother liquors afforded an additional 44 mg of isoatisine, m.p. 144–147°.

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