CONSTITUTION OF THE ALKALOID KESSELRINGINE*

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The homoproaporphine structure of kesselringine (*I*) having a half-ketal grouping between $C_{(1)}$ and $C_{(12)}$ was determined and its configuration derived.

From the corms of *Colchicum kesselringi* RGL (*C. regelii* STEF.), growing on the territory[1] of the Uzbek Soviet Socialist Republic, there was isolated[2,3] the non-tropolone alkaloid kesselringine, which on the basis of the elemental analysis and mass spectra was assigned[4,5] the formula $C_{19}H_{25}NO_4$. The purpose of our work was a further verification of the structure of that alkaloid and the determination of its relative and absolute configuration.

Ultraviolet spectroscopy[6] showed that kesselringine contains one aromatic ring. In the UV spectrum measured in alkaline ethanol, the bands at 292 nm and at 231 nm undergo a bathochromic shift by c. 10 nm and show hyperchromicity, which indicates the presence of a a free phenol group. According to the infrared spectrum (Fig. 1*a*), the alkaloid possesses hydroxyl groups (3555 and 3590 cm^{-1}) but no oxo groups. The ^{1}H-NMR spectrum of kesselringine (*I*) exhibits a singlet of the N-methyl group (δ 2·37), a singlet of the aliphatic methoxyl group (δ 3·38), a multiplet of one proton of the OCH type (δ 3·78) (width 8 Hz), and a singlet of the isolated aromatic proton (δ 6·51). In the mass spectrum of kesselringine, the molecular ion (*m/e* 331·1780, $C_{19}H_{25}NO_4$) eliminates a molecule of methyl-methyleneimine yielding the fragment *a* (*m/e* 288, $C_{17}H_{20}O_4$). The ion *a* loses further methanol (*m/e* 256, m* 227·6) or water (*m/e* 270, m* 253·1). The ion M − 1 (b.p. $C_{19}H_{24}NO_4$) also eliminates the molecule of methanol. The methoxyl group is evidently coupled to the sp^3 hybridized

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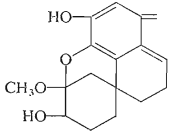
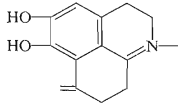
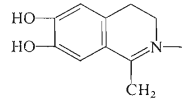
carbon atom. Furthermore, the mass spectrum shows nitrogen containing fragments *b* (*m/e* 230, $C_{14}H_{16}NO_2$) and *c* (*m/e* 191, $C_{11}H_{13}NO_2$); the former is accompanied by a satelite which is by two hydrogen atoms poorer. The other significant ions *d* and *e* have *m/e* 188 ($C_{12}H_{12}O_2$) and 173 ($C_{11}H_9O_2$). The presence of the fragments *a* – *e* indicates a homoproaporphine structure of the alkaloid as previously[4,5] suggested on the basis of chemical degradations.



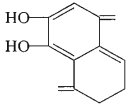
a



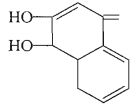
b



c



d



e

Labelling of kesselringine with C_2H_5OD in the ion source of the mass spectrometer increases the mass of its molecular ion by 2 amu, the masses of the ions *b* – *e* shift by 1 amu. Consequently, the key ion *b* arises by rupture of the oxygen bridge which connects the aromatic ring A with the spatially close ring D. Since the ^{1}H-NMR spectrum contains the signal of only one proton of the OCH type assigned to the proton geminal to the secondary alcohol group, this bridge must terminate at the quaternary carbon atom. Thus, the four oxygen atoms are present in the phenolic OH, the alcoholic OH, the CH_3O, and in the ethereal —O—. The molecule consists of five rings one of which is an aromatic one. From a comparison of the elemental composition of the ion *a*, *b*, and *d*, it is concluded that the secondary hydroxyl group is located in the six-membered ring D. The width of the multiplet (8 Hz) of the corresponding OCH proton in the ^{1}H-NMR spectrum indicates the vicinity of only one methylene group. Thus, its location in the position 10 is precluded. Since the ions *b* and *d* contain only two oxygen atoms, the positions 9 and 13 can also be eliminated so that only the $C_{(11)}$ and $C_{(12)}$ remain. When the ring D is present in an energetically more favourable chair conformation, then the OCH proton is in the *equatorial* position and the OH group in the *axial* position. In view of the above-mentioned arguments, it is also possible to locate the methoxyl group in the ring D.

On methylation with diazomethane, kesselringine (*I*) is converted to O-methylkesselringine ($C_{20}H_{27}NO_4$) (*II*). The UV spectrum in alkaline medium of the product *II* does not exhibit bathochromic or hyperchromic shift of the maxima of the longest wavelength bands. In view of these data, the new introduced methyl group can be localized in the ring A. The ^{1}H-NMR spectrum of the compound *II* contains a singlet of the N-methyl group at 2·50 ppm, singlets of two methoxyl groups at 3·43 and 3·83 ppm a one-proton multiplet of the OCH proton at 3·85 ppm, and a singlet of an isolated aromatic proton at 6·54 ppm. The nuclear Overhauser effect was observed between the methoxyl δ 3·83 and the singlet of the aromatic proton, which provides evidence of their mutual *ortho*-position. The doublet (J = 11·5 Hz) at 1·52 ppm can be interpreted as a low-field half of the AB system of the isolated methylene group. This also indicates that the oxygen bridge connects the hydroxyl at $C_{(1)}$ (ring A) with the position $C_{(12)}$ in the ring D. The chemical behaviour of kessel-

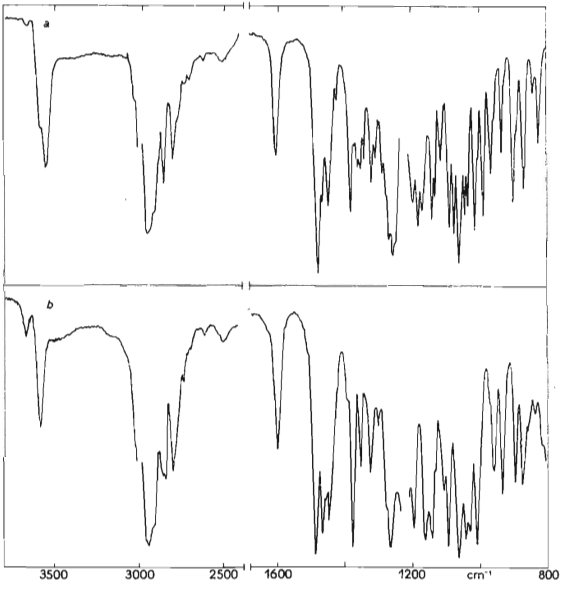
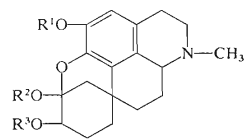


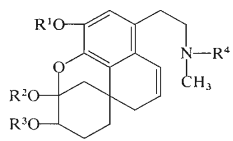
FIG. 1
Infrared Spectra (in chloroform) of Kesselringine (*I*) (*a*) and O-Methylkesselringine (*II*) (*b*)

ringine (*I*) and that of its methyl derivative *II* show that methoxyl is attached to the same carbon atom. The secondary alcohol group is then located at the vicinal $C_{(11)}$. The IR spectrum of O-methylkesselringine (*II*) (Fig. 1*b*) exhibits an OH band at 3580 cm^{-1} (in chloroform). In its mass spectrum, the ions *a* – *e* are shifted by 14 amu to higher values, in the same manner as the molecular peak. The ion *b* (*m/e* 244) is accompanied by a satellite of *m/e* 242 which is characteristic for homoproaporphines. The same ions are present in the mass spectrum of bulbocodine[7], they do not appear on the spectra of the proaporphine alkaloids of similar structure[8].

O-Methylkesselringine (*II*) is hydrolyzed in acidic medium at the original methoxyl group to the compound *IV*. The compound *IV* can also be prepared *via* the compound *VI* by acidic hydrolysis and methylation with diazomethane from kesselringine (*I*). The hydrolysis of the aliphatic methoxyl group cannot, however, be effected in alkaline medium, which indicates that the methoxyl group in compound *I* and that in O-methylkesselringine (*II*) are constituents of the ketal function. In acidic medium, the ketal methyl group can be replaced by a butyl group in *V*.

By the action of acetic anhydride on the compound *II*, the free hydroxyl group is acetylated (band at 1770 cm^{-1} in $CHCl_3$) and, simultaneously, the tetrahydroisoquinoline ring B is opened to give rise to the compound *III*. Treatment of the compound *II* or *IV* with acetyl chloride or acetic anhydride at higher temperature yields the triacetyl derivative *VIII* (IR bands at 1670 and 1770 cm^{-1} in nujol or 1630 and 1735 in KBr). The same compound arises on acetylation of regelamine[9]. Action of benzoyl chloride on kesselringine (*I*) gives rise to the tribenzoyl derivative *VII*.





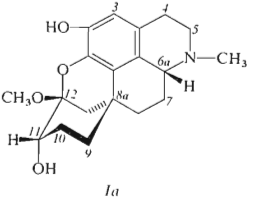
I, $R^1 = R^3 = H$; $R^2 = CH_3$
II, $R^1 = R^2 = CH_3$; $R^3 = H$
IV, $R^1 = CH_3$; $R^2 = R^3 = H$
V, $R^1 = R^3 = H$; $R^2 = C_4H_9$
VI, $R^1 = R^2 = R^3 = H$

III, $R^1 = R^2 = CH_3$; $R^3 = R^4 = COCH_3$
VII, $R^1 = R^3 = R^4 = COC_6H_5$; $R^2 = CH_3$
VIII, $R^1 = CH_3$; $R^2 = R^3 = R^4 = COCH_3$

The corresponding double bonds of the compounds *III*, *VII*, and *VIII* in the ring C could be demonstrated by UV and ^{1}H-NMR spectra[10]. In the mass spectrum of the substance *VIII* (M^+ 457), the fragments *m/e* 384 ($M - NH(CH_3)COCH_3$) and *m/e* 371 ($M - CH_2N(CH_3)COCH_3$) confirm the open character of the amine moiety. The ^{1}H-NMR spectrum of this compound *VIII* exhibits the signals of three acetyl groups and of one N-methyl group at 2·06, 2·11, and 2·25 (6 H) ppm, the singlet

of the methoxyl group at 3·82 ppm, a multiplet of two olefinic protons in the region between 5·42 to 6·03 ppm, and a singlet of an aromatic proton at 6·48 ppm. The signal of the proton of the OCH type is shifted downfield (5·36 ppm), which indicates the acetylation of the hydroxyl.

Consequently, kesselringine (*I*) is a homoproaporphine alkaloid with a free phenol group at $C_{(2)}$. Another phenolic hydroxyl at $C_{(1)}$ forms a constituent of the ketal grouping. Since the ketal group is located at $C_{(12)}$ (the ring D has a chair form), the secondary hydroxyl group must be (as results from the ^{1}H-NMR analysis[11] and in view of the above-mentioned observations) in the axial position and, consequently, in the quasi-*cis* position to the vicinal methoxyl group in the ketal grouping. Kesselringine (*I*) shows negative Cotton effects in its CD spectrum at 250 and 300 nm and a positive Cotton effect at 220 nm (^1L_b) (Fig. 2). From an analogy between proaporphines[12,13] and homoproaporphine[13] alkaloids, we assume for kesselringine the (6a*R*, 8a*S*, 11*S*, 12*R*) configuration according to the formula *Ia*:



Ia

The compound *IV* (12-demethyl-O-methylkesselringine) is probably identical with regelamine[9] (m.p. 226°C, $[\alpha]_D$ +33° (methanol)) and the compound *VI* (12-demethyl-

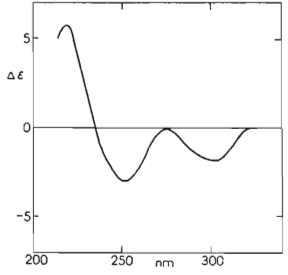


FIG. 2
CD Curve of Kesselringine (*I*) in Ethanol

kesselringine) with kesselridine[14] (m.p. 234°C, $[\alpha]_D$ −50° (pyridine)). Kesselringine (*I*) has also been dealt with in ref.[15]. Recently, Kametani and coworkers[16] synthesized a compound similar to O-methylkesselringine (*II*) in form of two isomers. Since, however, the authors do not report the IR spectra, it cannot be decided upon which of the two isomers is identical with natural O-methylkesselringine.

EXPERIMENTAL

The melting points have been determined on the Kofler block and are not corrected. The UV spectra were measured on a Unicam SP-700 in ethanol. The alkaline–ethanolic medium was prepared by adding 0·4 ml of 0·1M-NaOH to 2·50 ml of ethanolic solution. The IR spectra were measured on a Perkin-Elmer model 621, the ^{1}H-NMR spectra on a Varian model HA 100 (USA) or on a Jeol FX-60 (Japan), the mass spectra on an AEI-MS 902 (England) and the CD spectra on a Roussel-Jouan Dichrograph CD 185, model II (France).

Kesselringine (I)[2–5,15], m.p. 194–196°C, $[\alpha]_D^{22}$ +53° (*c* 3·0 in methanol), $[\alpha]_D^{22}$ +93° (*c* 1·0 in chloroform). UV: λ_{max} 231 nm (log ε 3·85), 293 (3·44) (in ethanol); λ_{max} 224 nm (log ε 4·30), 255(3·76), 305 (3·56) (in ethanol + NaOH). CD data: ethanol −293 nm ($\Delta\varepsilon$ −1·86), 285 i (−1·67), 241 (−3·05), 218 (+5·80), negative at short wavelengths; (ethanol + 1% HCl conc.) −293 (−1·39), 285 i (−1·31), 245 (−0·55), 228 (+0·89), negative at short wavelengths. M^+ 331 (40, $C_{19}H_{25}NO_4$), 330 (100), 316 (4·2), 314 (2·7), 298 (6·7, $C_{18}H_{20}NO_3$), 288 (41, $C_{17}H_{20}O_4$), 270 (4·4, $C_{17}H_{18}O_3$), 256 (16, $C_{16}H_{16}O_3$), 242 (7·1, $C_{15}H_{16}NO_2$), 230 (23, $C_{14}H_{16}NO_2$), 228 (12, $C_{14}H_{14}NO_2$), 216 (6·7, $C_{13}H_{14}NO_2$), 214 (8·0), 202 (8·0, d 2 : 1, $C_{12}H_{12}NO_2$, $C_{13}H_{16}NO$), 191 (8·9, $C_{11}H_{13}NO_2$), 188 (10, $C_{12}H_{12}O_2$), 173 (7·1), 115 (10), 91 (6·7), 77 (6·0).

O-*Methylkesselringine* (II). To the methanolic solution of (*I*), diazomethane in hexane was added in excess. After removal of the solvent, the compound *II* crystallized from acetone, m.p. 200°C. UV: λ_{max} 212 nm (log ε 4·56), 234 (3·83) s, 287 (3·41) s, 292 (3·43); the same spectrum was obtained in ethanol + NaOH. MS (*m/e*, relative intensity, composition): M^+ 345 (46), 344 (100, $C_{20}H_{26}NO_4$), 330 (4·6), 314 (2·4), 303 (11), 302 (53, $C_{18}H_{22}O_4$), 284 (2·3), 270 (6·5, $C_{17}H_{18}O_3$), 256 (2·2), 245 (2·8), 244 (11, $C_{15}H_{18}NO_2$), 242 (6·5), 230 (2·6), 229 (2·4), 228 (3·4), 215 (2·8), 214 (2·4), 213 (2·4), 205 (3·8, $C_{12}H_{15}NO_2$), 203 (2·4), 202 (15, $C_{13}H_{14}O_2$), 201 (2·7), 200 (4·9), 187 (4·3, $C_{12}H_{11}O_2$), 143 (3·0), 142·5 (11), 142 (2·4), 42 (3·2). The mother liquors were concentrated in vacuum and the residue crystallized from acetone to give the quaternary base.

N,O-*Diacetyl*-O-*methylkesselringine* (III). A mixture of *II* (200 mg), acetic anhydride (2 ml) and anhydrous sodium acetate (0·8 g) was heated at 45°C for 48 h. After removal of the rest of acetic anhydride, the residue was dissolved in water and extracted with chloroform, evaporated and dried in vacuum to yield amorphous *III*. IR (nujol): λ_{max} 1 670 cm^{-1} (amide group), 1 770 cm^{-1} (ester).

12-*Demethyl*-O-*methylkesselringine* (IV). A solution of *II* (200 mg) in 7% sulphuric acid (5 ml) was heated on a water bath for 2 h. After cooling, the solution was made alkaline with ammonia and extracted with chloroform to afford the compound *IV*, m.p. 223–224°C (acetone). ^{1}H-NMR: absence of the signal of the OCH_3 group at 3·42 ppm. MS: M^+ 331 (41, $C_{19}H_{25}NO_4$), 330 (100), 289 (7·4), 288 (36, $C_{17}H_{20}O_4$), 270 (7·4), 244 (26, $C_{15}H_{18}NO_2$), 242 (16).

12-*Butyl*-12-*demethylkesselringine* (V). A solution of *I* (500 mg) in butanol (20 ml), containing 7% of conc. hydrochloric acid, was heated for 1 h, evaporated in vacuum, the residue dissolved in water, made alkaline with ammonia, and extracted with chloroform. After evaporation, the extract gave the compound *V*, m.p. 160–161° (acetone). MS: M^+ 373.

12-*Demethylkesselringine* (VI). A solution of *I* (500 mg) in 7% sulphuric acid (10 ml) was heated for 2 h. After cooling, the solution was made alkaline with ammonia and extracted with chloroform. After evaporation, the residue crystallized from a mixture of acetone and water, m.p. 236–237°C. ^{1}H-NMR: absence of the OCH_3 signal. MS: M^+ 317 (40, $C_{18}H_{23}NO_4$), 316 (100), 275 (5·7), 274 (32), 256 (8·6), 230 (12), 228 (10). By the reaction of diazomethane in methanol, the compound *VI* was converted into the compound *IV*.

N,O,O-*Triacetyl*-12-*demethyl*-O-*methyl*-*kesselringine* (VIII). A mixture of *IV* (100 mg), acetic anhydride (2 ml) and anhydrous potassium acetate (500 mg) was left standing at 70°C for three days and then heated on a boiling water bath for 3 h. to afford *VIII*, m.p. 140–142°C (acetone)[9]. Yield 70%. A small quantity (5%) of the compound *VIII* was also obtained from the mother liquors after the crystallization of *III*. UV: λ_{max} 210 nm (log ε 4·73), 236 (4·19), 273 (3·88), 280 (3·88), 302 (3·37), 314 (3·33). IR (KBr): λ_{max} 1625 (C=C), 1640 ($N(CH_3)COCH_3$), 1740 cm^{-1} (CH_3COO). MS: M^+ 457, 415, 397, 384, 342, 329, 324, 282, 269.

Tribenzoylkesselringine (VII). To a solution of *I* (500 mg) in 20% sodium hydroxide (5 ml), benzoyl chloride (1 ml) was added and the mixture stirred for 3 h, extracted with chloroform, washed with 1% sodium hydroxide, 1% hydrochloric acid and water. After drying and evaporation, the yield gave the compound *VII*, m.p. 157–159°C (acetone–water) (after removal of crystal water, m.p. 105°C).

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