UDC 547.944/945

# THE STRUCTURE OF PERFORINE AND HAPLOPHYLLIDINE

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Khimiya Prirodnykh Soedinenii, Vol. 4, No. 6, pp. 360-367, 1968

Isolation from seeds of Haplophyllum perforatum of the new alkaloids perforine and haplophyllidine has been reported previously [1, 2]. Perforine (I), $C_{18}H_{25}O_5N$, is a weak optically-active base, a functional analysis of which shows that it contains two hydroxy and two methoxy groups and no N—$CH_3$ group.

The IR spectrum of perforine has bands characteristic for substances containing a furan group [3]. The developed formula of the base is $C_{16}H_{17}N\ (OCH_3)_2(OH)_2(-O-)$.

The presence of a hydroxy group in (I) is confirmed by the preparation of a chloroacetyl derivative (IV).

$$C_{16}H_{17}N(COH_3)_2\ (OCOCH_3)\ (Cl)\ (—O—).$$

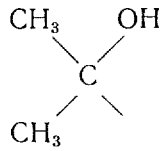
Frequency of the carbonyl absorption of (IV) (1740 $cm^{-1}$), the replacement of a second hydroxy group by chlorine under the action of acetyl chloride on perforine, and the insolubility of the alkaloid in alkali show that both hydroxy groups are alcoholic and one of them is tertiary. The preparation of a ketone (XI) by the oxidation of perforine with chromic anhydride in glacial acetic acid shows the secondary nature of the other hydroxy group in (I).

The action of (I) of zinc and hydrochloric acid forms anhydroperforine (III), $C_{18}H_{23}O_4N$, and a chlorine-containing substance (V), $C_{17}H_{19}O_2NCl_2$, which are also obtained by the action of anhydrous zinc chloride on perforine. Anhydroperforine (III) does not undergo acetylation. Its IR spectrum lacks the absorption bands of OH and CO groups, and, consequently, the splitting out of water takes place at the expense of the two hydroxy groups with the formation of an ether bridge. Thus, the developed formula of anhydroperforine can be represented in the following way:

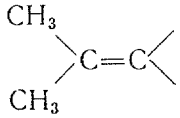
$$C_{16}H_{17}N\ (OCH_3)_2\ (—O—)_2.$$

The oxidation of perforine (I) and anhydroperforine (III) by the Kuhn-Roth method gives acetone.

All these data show the presence in perforine (I) of the grouping



Boiling chloroacetylperforine (IV) with pyridine leads to the splitting out of hydrogen chloride and the formation of a substance (VI) identical with acetylhaplophyllidine [2]. When the latter is saponified, haplophyllidine (II) is formed. The passage from perforine to haplophyllidine shows that these alkaloids have the same heterocyclic skeleton. Their difference is that substance (II) contains an isopropylidene grouping



in place of the dimethly carbinol group in (I). This is shown by the production of acetone when haplophyllidine is subjected to ozonolysis [4]. The oxidation of (II) by the Kuhn-Roth method also gives acetone. The composition of (II), $C_{18}H_{23}O_4N$, is confirmed by a mass-spectrometric determination of its molecular weight and by the number of protons in its NMR spectrum.

The UV spectra of (I)–(III) are almost identical (table), but they differ from the UV spectra of the furoquinoline alkaloids.

The IR spectra of (I) and (II) have absorption bands characteristic for the furan ring, the presence of which has been shown by the catalytic hydrogenation of these substances. Under these conditions perforine gives tetrahydroperforine (VII), $C_{18}H_{29}O_5N$, and haplophyllidine gives tetrahydrohaplophyllidine and hexahydrohaplophyllidine (VIII), $C_{18}H_{29}O_4N$. The IR spectra of (VII) and (VIII) exhibit the absorption band of an amide carbonyl (1640 $cm^{-1}$). Consequently, the furan ring is condensed linearly with the pyridine ring in (I) and (II). The UV spectra of (VII) and (VIII) are similar to those of the 2-pyridones [5].

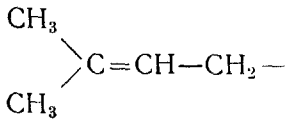
The hydrogenation of perforine in the presence of Raney nickel gives dihydroperforine (IX). Its UV spectra is similar to that of fabianine, which has a 5,6,7,8-tetrahydroquinoline structure [6].

The similarity of the UV spectra of the products of the hydrogenation of (VII) and (VIII) to the corresponding spectra of 2-pyridone derivatives and of the spectra of dihydroperforine (IX) to those of 5,6,7,8-tetrahydroquinoline derivatives, and also the mass spectrometric data of a study of haplophyllidine permit the conclusion that the alkaloids (I) and (II) are based on a 5,6,7,8-tetrahydro-2,3-furoquinoline structure.

Results of a study of the NMR spectra confirm this hypothesis. In the aromatic region in the NMR spectra of (I)–(III) there are only two one-proton doublets at $\tau$ 2.53–2.61 and 3.13–3.24 (J = 3 Hz), the chemical shifts and spin-spin coupling constants of which are typical for the $\alpha$- and $\beta$-protons of a furan ring [7]. There are no signals of other aromatic protons.

The NMR spectrum of (I)–(III) have two three-proton singlets in the $\tau$ 5.78–5.85 and 6.92–6.95 regions corresponding to two methoxy groups, the signal in the weaker field relating to a 4-OMe group in a pyridine ring and that in the stronger field to a methoxy group in a hydrogenated ring.

The presence in the spectrum of (II) of a one-proton triplet at $\tau$ 4.72 (J = 7 Hz) and of two three-proton singlets at $\tau$ 8.27 and 8.33 shows the presence in this base of the side chain



The strong peaks of ions with m/e 248 in the mass spectra of (II) and (VI) show the splitting off to a side chain of such a structure from haplophyllidine.

The oxidation of haplophyllidine with chromic anhydride in glacial acetic acid with the consumption of three

UV Spectra in Ethanol

| Compound | $\lambda_{max}$, mμ (log ε) | $\lambda_{min}$, mμ (log ε) |
|---|---|---|
| Perforine (I) | 256 (4.00) | 233 (3.58) |
| Haplophyllidine (II) | 258 (4.18) | 234 (3.68) |
| Anhydroperforine (III) | 258 (4.08) | 233 (3.64) |
| (IV) | 219, 258 (4.52; 4.14) | 232 (3.68) |
| (VII) | 233, 302 (3.64; 3.92) | 228, 253 (3.62; 2.88) |
| (VIII) | 236, 300 (3.64; 3.93) | 230, 253 (3.62; 3.04) |
| (IX) | 210, 278 (4.38; 3.76) | 243 (3.40) |
| (XII) | 233, 282, 292, 318 (4.18; 4.10; 4.12; 4.27) | 220, 263, 300 (4.04; 3.82; 4.08) |
| (V) | 234, 252, 280, 296, 319 (4.08; 4.02; 4.05; 4.06; 4.30) | 220, 264, 300 (3.93, 3.78, 4.04) |
| (XIII) | 255, 262, 354 (3.66; 3.63; 4.26) | 240, 270, 295 (3.62; 3.48; 3.20) |
| (XIV) | 212, 252, 302, 336 (4.04; 4.18; 4.19; 4.10) | 229, 266 (3.72; 3.70) |
| (XV) | 219, 295, 315 (4.4; 4.26; 4.32) | 260, 302 (3.67; 4.21) |

oxygen atoms gives a lactone (X), $C_{15}H_{15}O_5N$. The absorption band at 1790 $cm^{-1}$ in its IR spectrum shows that it is a $\gamma$-lactone and not a $\delta$-lactone [8]. This means that the hydroxy group and the side chain in (I) and (II) must be present on adjacent carbon atoms of the tetrahydroquinoline nucleus.

The absorption band at 1740 $cm^{-1}$ in the IR spectrum of the ketone (XI) shows that the carbonyl group in it is conjugated with the pyridine ring, and, therefore, the hydroxy group in (I) and (II) may assume position 6 or 7.

The NMR spectra of (I) and (II) contain a one-proton signal in the $\tau$ 5.85–6.24 region the chemical shift of which corresponds to a proton in the neighborhood of a hydroxy group [9, 10]. The shift of this signal in the spectrum of (VI) ($\tau$ = 4.72) shows the correct assignment of the signal and the secondary nature of the hydroxy group located in the ring.

The absence of a signal from a proton adjacent to a methoxy group [10] shows that the $OCH_3$ group is located on a tertiary carbon atom, i.e., on the same carbon atom as the side chain.

The action of mineral acids on (I)–(III) forms a substance (XII), $C_{17}H_{19}O_3N$, the composition of which was confirmed by mass-spectroscopic data and also by the results of a determination of the number of protons in its NMR spectrum. Compound (XII) lacks hydroxy and carbonyl groups but has one methoxy group: $C_{16}H_{16}N(OCH_3)(-O-)_2$.

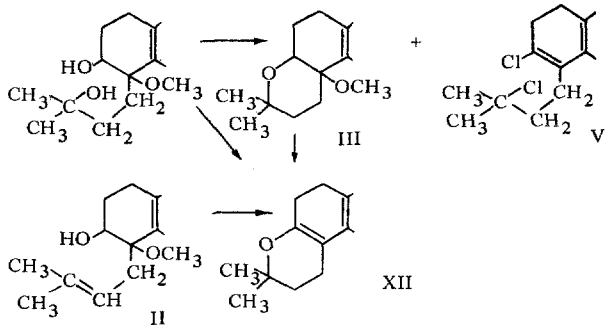
The IR spectrum of (XII) has the C—H stretching vibrations of a furan ring which disappear when this compound is hydrogenated.

Compound (XII) differs in composition from haplophyllidine (II) by $CH_3OH$ and from perforine (I) by $CH_3OH$ and $H_2O$. In this reaction, the formation of a new ring takes place (in perforine by the splitting out of a molecule of water from two hydroxy groups and in haplophyllidine by the transition from a chain isomer to a ring isomer [11]).

Splitting out of a methoxy group in the form of methanol leads to the formation of a double bond conjugated with the pyridine ring, since in the IR spectrum of (XII) there is a bathochromic shift, in comparison with the corresponding spectra of (I)–(III), accompanied by a hyperchromic effect.

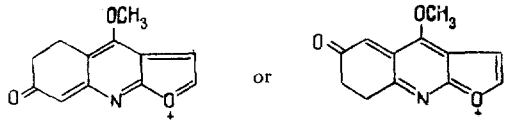
This ease of splitting off of a methoxy group under the action of acids with the formation of a conjugated double bond shows that the $OCH_3$ group and, consequently, the side chain occupy position 5 or 8 of the tetrahydroquinoline nucleus.

The NMR spectrum of (XII) has no signals of olefinic protons, which means that the double bond in this compound is tetrasubstituted.



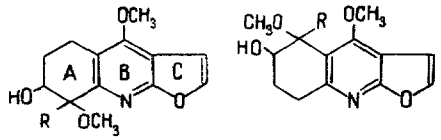

Product (XII) undergoes the conversion into the iso compound $C_{17}H_{19}O_3N$ [12], that is characteristic for 4-alkoxyquinolines, like many 4-quinolones, firmly retaining a molecule of water [13]. The developed formula of the iso product (XIV) is $C_{15}H_{16}(N-CH_3)(-O-)_2(CO)$. Under similar conditions, the bases (I)–(III) give substance (XIV).

In the mass spectrum of (II) there are intense peaks of ions with m/e 285 and 216 corresponding to the expulsion from the molecular ion first of a molecule of $CH_3OH$ and then of the side chain. The stability of the ion (M-101) is due to the formation of a system of conjugated double bonds:




m/e 216 (M-101)

On the basis of all that has been said above, the following structures may be proposed for (I) and (II):



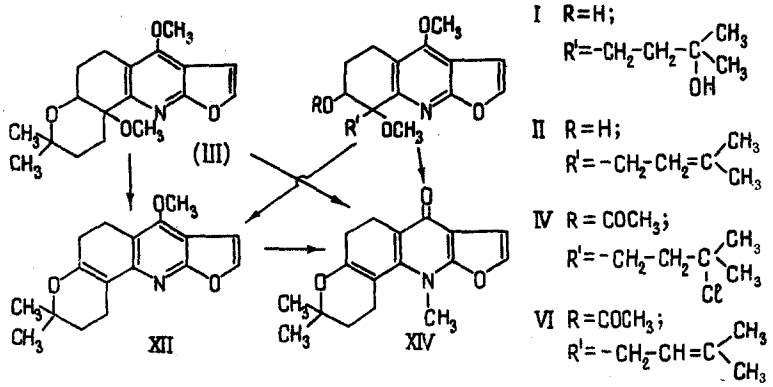

The action of concentrated sulfuric acid on (I) and (II) gives an optically inactive base $C_{17}H_{15}O_2N$ (XV). Its mass spectrum has a 100% molecular ion, which is characteristic for aromatic systems.

In the NMR spectrum of (XV) in the region of aromatic protons, in addition to signals from the $\alpha$- and $\beta$-protons of a furan ring, there are two doublets at $\tau$ 1.85 and 2.97 ($J_{ortho}$ = 8 Hz). The values of the chemical shifts of aromatic protons [7] make it possible to consider formula *a* as more likely.

Below we give the scheme of the transformations of perforine (I) and haplophyllidine (II) (see figure on p. 773).

## Experimental

Perforine (I). This substance was obtained with mp 182–183° C (methanol), $[\alpha]_D^{20}$ + 14.56° (c 1.579; methanol), $[\alpha]_D^{20}$ + 14.32° (c 0.3044; chloroform). IR spectrum: 3380–3470 $cm^{-1}$, 3148, 3118, 1610, 1267, 1100, 875 $cm^{-1}$.




Found, %: C 64.4, 64.5; H 7.70, 7.61; N 4.27, 4.42; $OCH_3$ 18.49, 18.77. Calculated for $C_{18}H_{25}O_5N$, %: C 64.45; H 7.51; N 4.17; $OCH_3$ 18.5.

Anhydroperforine (III). Several small pieces of zinc were added to a solution of 1 g of perforine in 12 ml of concentrated hydrochloric acid, and the mixture was left to stand at room temperature. After 5 days, it was filtered and made alkaline with ammonia and the precipitate that deposited was treated with chloroform. The oil remaining after the solvent had been distilled off crystallized on the addition of ethanol. The yield of anhydroperforine was 0.7 g, mp 143.5–144° C (ethanol), $[\alpha]_D^{20}$ –36.02° (c 2.208; methanol); IR spectrum: 3130 $cm^{-1}$, 1610, 1265, 1100, 878 $cm^{-1}$.

Found, %: C 68.18, 68.18; H 7.35, 7.34; N 4.59, 4.47; $OCH_3$ 19.3, 18.8; mol. wt. 317 (mass spectrometry), Calculated for $C_{18}H_{23}O_4N$, %: C 68.11; H 7.3; N 4.41; $OCH_3$ 19.5; mol. wt. 317.37.

From the mother liquor, after the isolation of the anhydroperforine, a chlorine-containing substance (V) was obtained with mp 217-218° C (from ethanol); $[\alpha]_D$ 0°.

Found, %: $OCH_3$ 8.5, 8.2; Cl 20.0, 20.0. Calculated for $C_{17}H_{19}O_2NCl_2$, %: $OCH_3$ 9.1; Cl 20.8.

Chloroacetylperforine (IV). A mixture of 1 g of perforine and 10 ml of acetyl chloride was left in a sealed tube at room temperature. After 7 days, the tube was opened and the excess of acetyl chloride was evaporated off. The residue consisted of an oil which crystallized on the addition of water, mp 157–158° C (ethanol); $[\alpha]_D^{18}$ –21.3° (c 2.490; chloroform); IR spectrum: 3145 $cm^{-1}$, 3120, 1730 $cm^{-1}$.

Found, %: Cl 8.74, 8.60. Calculated for $C_{20}H_{26}O_5NCl$, %: Cl 8.97.

Acetylhaplophyllidine (VI). A solution of 1 g of chloroacetylperforine in 10 ml of dry pyridine was boiled for 8 hr [14]. Then the solution was acidified and extracted with chloroform, the distillation of which yielded 0.6 g of technical acetylhaplophyllidine with mp 145–146° C (methanol).

Haplophyllidine (II). The substance was formed by the saponification of acetylhaplophyllidine with alcoholic alkali, mp 110–111° C.

Found, %: C 68.20, 68.10; H 7.37, 7.49; N 4.40, 4.46; mol. wt. 317 (mass spectrometry). Calculated for $C_{18}H_{23}O_4N$, %: C 68.11; H 7.3; N 4.41; mol. wt. 317.37.

Ozonolysis of haplophyllidine. Ozone was passed through a solution of 0.4 g of haplophyllidine in 3 ml of glacial acetic acid for 1.5 hr. The solution was treated with 10 ml of water and boiled for 10 min, and then 16.5 ml of 2 N caustic soda solution and 5 ml of 1 N potassium permanganate solution were added. The reaction mixture was distilled with steam. The distillate was collected in a solution of 2,4-dinitrophenylhydrazine hydrochloride. A precipitate deposited with mp 123–124° C (ethanol) which gave no depression of the melting point with acetone 2,4-dinitrophenylhydrazone.

Kuhn-Roth oxidation of perforine. 0.5 g of perforine was oxidized with a mixture of 5 g of chromic anhydride, 5 ml of concentrated sulfuric acid, and 20 ml of water. The volatile reaction products were trapped in a 0.1% solution of 2,4-dinitrophenylhydrazine hydrochloride. A precipitate of acetone 2,4-dinitrophenylhydrazone with mp 123–124° C deposited.

Under similar conditions, anhydroperforine and haplophyllidine also gave acetone.

Tetrahydroperforine (VII). 0.5 g of perforine in 10 ml of ethanol was reduced with the platinum catalyst prepared from 0.2 g of platinum oxide. After 1 hr, 75 ml of hydrogen had been absorbed. The catalyst was filtered off with

suction and washed with ethanol. After the ethanol had been distilled off, an oil remained which crystallized on trituration with absolute ether. Mp 105–107° C (absolute ether); $[\alpha]_D^{21}$ + 16.1° (c 1.301; ethanol). IR spectrum: 1640 $cm^{-1}$.

Found, %: N 3.86, 3.96; $OCH_3$ 18.56, 18.09. Calculated for $C_{18}H_{29}O_5N$, %: N 4.13; $OCH_3$ 18.3.

Hexahydrohaplophyllidine (VIII). 1.3 g of haplophyllidine in 15 ml of glacial acetic acid was hydrogenated over the platinum catalyst prepared from 0.2 g of platinum oxide. Hydrogenation took 12 hr and 320 ml of hydrogen was absorbed. The catalyst was separated off and the filtrate was evaporated at room temperature. The residue was dissolved in water, the solution was made alkaline with ammonia, and the base that deposited was crystallized from acetone, mp 146–147° C. This gave a depression of the melting point with tetrahydrohaplophyllidine [2]. IR spectrum: 1645 $cm^{-1}$.

Found, %: C 67.4; H 8.81; N 4.4. Calculated for $C_{18}H_{29}O_4N$, %: C 66.8; H 8.98; N 4.33.

Dihydroperforine (IX). In the presence of Raney nickel, 0.1 g of perforine in 50 ml of ethanol was hydrogenated for 3 hr, 5 ml of hydrogen being absorbed. The catalyst was filtered off with suction and washed with ethanol to give 0.08 g of dihydroperforine with mp 169° C.

Oxidation of haplophyllidine with chromic anhydride. A solution of 1 g of haplophyllidine in 10 ml of glacial acetic acid was treated with 0.63 g of chromic anhydride in 20 ml of glacial acetic acid. The mixture was left at room temperature until the solution had acquired a green color, after which it was extracted with ether. The ethereal extract, after being washed with water and 4% sodium carbonate solution, yielded substance (X) with mp 153–154° C (methanol); IR spectrum: 1790 $cm^{-1}$.

Found, %: N 4.69, 4.49. Calculated for $C_{15}H_{15}O_5N$, %: N 4.84.

Oxidation of perforine with chromic anhydride. A solution of 0.3 g of chromic anhydride in 15 ml of glacial acetic acid was added to 0.5 g of perforine in 5 ml of glacial acetic acid. The mixture was left at room temperature for 48 hr. The ether extract of the solution, after dilution with water (20 ml), and the distillation of the ethereal extract after its washing with 4% sodium carbonate solution gave a technical product which formed a 2,4-dinitrophenylhydrazone with mp 138–140° C; IR spectrum: 1740 $cm^{-1}$.

The product (XII). A solution of 1 g of perforine in 10 ml of 25% sulfuric (or hydrochloric) acid was heated in the water bath for 2.5 hr. The ethereal solution obtained by extraction of the mixture made alkaline with 25% ammonia yielded, by distillation of the solvent, a substance with mp 125–126° C (ethanol); $[\alpha]_D^{21}$ + 9.04° (c 6.194; chloroform); IR spectrum: 1655 $cm^{-1}$, 1600, 3145, 3123 $cm^{-1}$.

Found, %: C 70.5, 70.7; H 8.04, 8.00; N 4.92, 4.86; $OCH_3$ 10.6, 10.5. Calculated for $C_{17}H_{23}O_3N$, %: C 70.5; H 8.01; N 4.84; $OCH_3$ 10.7.

Substances (II) and (III) formed (XII) under the same conditions.

Hydrogenation of XII. 0.5 g of substance (XII) with mp 126° C in 10 ml of ethanol was hydrogenated over a platinum catalyst. After 1 hr, 85 ml of hydrogen had been absorbed; the catalyst was filtered off with suction and washed with ethanol. Distillation of the filtrate yielded substance (XIII) with mp 225–226° C (ethanol); $[\alpha]_D^{21}$ ± 0°.

Found, %: C 70.5, 70.7; H 8.04, 8.00; N 4.92, 4.86; $OCH_3$ 10.6, 10.5. Calculated for $C_{17}H_{19}O_3N$, %: C 70.5; H 8.01; N 4.84; $OCH_3$ 10.7.

Product (XIII) was also obtained by the action of 25% sulfuric (or hydrochloric) acid on tetrahydroperforine (VII) and tetrahydrohaplophyllidine. IR spectrum: 1655, 1630 $cm^{-1}$.

Methiodide of (XII). In a sealed tube, 0.5 g of substance (XII) in a mixture of 3 ml of methanol and 5 ml of methyl iodide was heated in the boiling water bath for 20 hr. The crystals that deposited on cooling were separated off (0.3 g): mp 221–222° C (methanol). Found, %: I 30.39, 30.51. Calculated for $C_{17}H_{23}O_3N \cdot CH_3I$, %: I 29.

Isomerization of (XII) into (XIV). A solution of 0.6 g of the methiodide of (XII) in 10 ml of methanol was treated with 10 ml of 30% alcoholic potash and the mixture was boiled for 3 hr. The dry residue obtained after the evaporation of the solvent was washed with water. This gave 0.3 g of substance (XIV) with mp 168-169° C (acetone); $[\alpha]_D$ + 13.5° (c 0.884; ethanol); IR spectrum: 3140, 1645 $cm^{-1}$.

Found, %: C 67.3, 67.4; H 7.03, 6.94; N 4.75, 4.88; N–$CH_3$ 10.05, 10.44. Calculated for $C_{17}H_{19}O_3N \cdot H_2O$, %: C 67.3; H 6.97; N 4.62; N–$CH_3$ 9.5.

The same compound (XIV) was obtained in a similar manner from perforine, halophyllidine, and anhydroperforine.

Action of concentrated sulfuric acid on perforine. Fifteen milliliters of concentrated sulfuric acid cooled with a mixture of ice and salt, was added to 0.8 g of perforine (or haplophyllidine). The solution was left at room temperature for 1 hr. Then, it was poured into 150 ml of ice water and extracted with chloroform. Distillation of the solvent yielded

0.75 g of substance (XV) with mp 125–126° C (from ethanol). A mixture with compound (XII) gave a depression of the melting point. IR spectrum: 3145 $cm^{-1}$. 3120, 1005, 1240, 1090, 870, 833 $cm^{-1}$.

Found, %: C 77.1, 77.1; H 5.30; 5.50; N 5.33, 5.24; $OCH_3$ 11.35, 11.19; mol. wt. 265 (mass spectrometry). Calculated for $C_{17}H_{15}NO_2$, %: C 77.0; H 5.66; N 5.28; $OCH_3$ 11.7; mol wt. 265.30.

## Conclusions

1. The alkaloids perforine and haplophyllidine isolated from the seeds of H. perforatum have the developed formulas $C_{16}H_{17}N(OH)_2(OCH_3)_2(-O-)$ and $C_{16}H_{16}N(OH)(OCH_3)_2(-O-)$.

2. Perforine is 7-hydroxy-8-(3'-hydroxy-3'-methylbutyl)-4,8-dimethoxy-5,6,7,8-tetrahydrofuroquinoline, and haplophyllidine is 7-hydroxy-4,8-dimethoxy-(3'-methyl-2'-butenyl)-5, 6, 7, 8-tetrahydrofuroquinoline.

3. The transition from perforine to haplophyllidine has been effected by the splitting off of hydrogen chloride from chloroacetylperforine.

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9 November 1967



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