OXYNEMORENSINE, AN ALKALOID FROM *Senecio nemorensis* L., VAR. *subdecurrens* GRISEB.*

Antonín KLÁSEK[a], Petr SEDMERA[b], Jindřich VOKOUN[b], Anna BOEVA[c], Svatava DVOŘÁČKOVÁ[d] and František ŠANTAVÝ[d]

[a] *Department of Leather Technology, Faculty of Technology, Technical University, 762 72 Gottwaldov,*
[b] *Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague,*
[c] *Faculty of Pharmacy, University of Sofia, 1000 Sofia, Bulgaria and*
[d] *Institute of Chemistry, Medical Faculty, Palacký University, 775 15 Olomouc*

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From *S. nemorensis* L., var. *subdecurrens* GRISEB. there were isolated the previously obtained alkaloids nemorensine (*I*), retroisosenine (*II*), bulgarsenine (*III*) and, in addition, the alkaloid oxynemorensine which was assigned the structure *VIII* on the basis of the interpretation of the ^{1}H-NMR, ^{13}C-NMR, mass spectra, and on that of the identification of the products of hydrolysis and reduction. Furthermore, the isolation of the *cis*-nemorensic acid (V) as well as that of the unsaturated acid *IV*, and the transformation of bulgarsenine (*III*) to nemorensine (*I*) were described.

In our previous paper[1], the isolation of the alkaloid nemorensine (*I*) from three varieties of *Senecio nemorensis* L., *i.e.* from var. *subdecurrens* GRISEB., ssp. *Jacquinianus* (RCHB.) DURAND and from ssp. *fuchsii*, var. *nova* (ZLATNIK) was reported. Later on, we succeeded to isolate[2] two other alkaloids – retroisosenine (*II*) and bulgarsenine (*III*) from *S. nemorensis* L., var. *bulgaricus* (VEL) STOJ. et STEF. A characteristic feature of all these isolated alkaloids is the exceptional structure with a thirteen-membered macrocycle. In the papers[1,2] we have already described that, in addition to the isolated alkaloids, the extract contains smaller quantities of at least two other alkaloids. In order to obtain them we have now worked up a larger quantity of roots of *S. nemorensis* L., var. *subdecurrens* GRISEB. The methanolic root extract was separated into portions containing acid substances, free alkaloids, and alkaloids present as their N-oxides.

On crystallization, the portion containing acidic substances yielded an unsaturated acid *IV* which was isolated from this plant earlier[1]. Column chromatography of the

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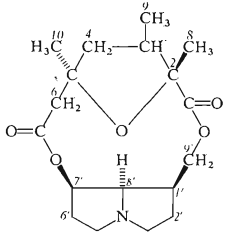
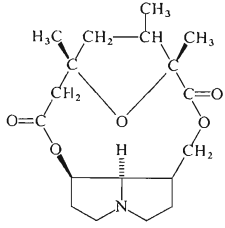
mother liquors on silica gel also gave the unsaturated acid *IV* and, in addition, a saturated acid which on the basis of the IR and ^{1}H-NMR spectra was identical with *cis*-nemorensic acid (*V*) obtained[2] by hydrolysis of the alkaloid retroisosenine (*II*). Furthermore thin layer chromatography showed that the mixture contained nemorensic acid (*VI*) which, however, could not be isolated from the mixture in pure state. All these acids form acid components of the so far isolated alkaloids nemorensine (*I*), retroisosenine (*II*), and bulgarsenine (*III*). To our knowledge, this is for the first time that from the plants of the genus *Senecio* there have been isolated native acids identical with those present in the alkaloids.

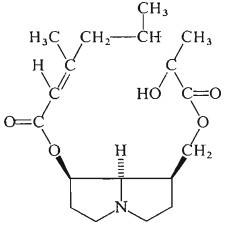
Since the attempt to prepare the unsaturated acid *IV* by alkaline hydrolysis of bul-



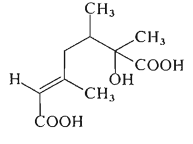
I



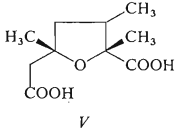
II



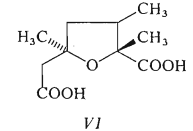
III



IV



V



VI

garsenine (*III*) failed giving instead the nemorensic acid (*VI*), we tried the conversion of the acid *IV* to nemorensic acid (*VI*) under conditions analogous to those of the hydrolysis of bulgarsenine (*III*). A mixture of substances was obtained whose major part was formed by the unreacted acid *IV*. Chromatographic separation of the mixture gave, in addition, *cis*-nemorensic acid (*V*). It is of interest that on alkaline hydrolysis of bulgarsenine (*III*), the conversion of the unsaturated acid *IV* to nemorensic acid (*VI*) proceeds quantitatively, whereas under the same conditions reaction of the free acid *IV* yields only a small quantity of the cyclic acid, *i.e.* the isomer *V* with a reversed configuration. The different results can be accounted for only by the fact that on hydrolysis of bulgarsenine (*III*) conversion of the acids *IV* → *VI* takes place by stereospecific intramolecular addition of the $C_{(1)}$—OH group to the double bond in the positions 5, 6 at the stage when the macrocycle is open only on one side (*i.e.* between the carboxyl at $C_{(1)}$ and the hydroxyl at $C_{(9')}$ because the allyl ester bond of the macrocycle is more easily hydrolyzable[3]).

The portion containing free alkaloids was purified and then repeatedly chromatographed, giving the three previously found[1,2] alkaloids nemorensine (*I*), retroisosenine (*II*), and bulgarsenine (*III*) and, furthermore, small quantities of two other alkaloids, *i.e.* the amorphous alkaloid SN-D, whose presence in the studied plants had already been described earlier[1], and the crystalline alkaloid SN-E which we named oxynemorensine.

The IR spectrum of oxynemorensine exhibits a large band of carbonyls at 1732 cm^{-1} (chloroform), and 1705 cm^{-1} (KBr), respectively, but no bands corresponding to double bonds and hydroxyl groups. Oxynemorensine does not absorb in the ultraviolet region of the spectrum, which indicates[4] the presence of the saturated acidic and basic moieties. In the mass spectrum, the elemental constituents of the molecular ion are $C_{18}H_{27}NO_6$ (*m/z* 357). Prominent peaks appear at *m/z* 337, 237, 222, 210, 183, 138, 123, 122, 119, and 82. The last four ions are of diagnostic value[5,6] for saturated necine bases. The ^{1}H-NMR spectrum shows a doublet of the secondary methyl at 0·98 ppm (J = 6·3 Hz), singlets of two tertiary methyl groups at 1·27 and 1·37 ppm, a doublet of doublets at 4·14 ppm (J = 0·5 and 12·7 Hz), a doublet of doublets at 4·58 ppm (J = 3·4 and 12·7 Hz), and a multiplet at 5·46 ppm ($W_{1/2}$ = = 8·8 Hz). In the envelope of methine and methylene protons, the double resonance experiment revealed a one-proton triplet at 1·69 ppm (J = 10·7 Hz) and one AB-system (2·33 and 2·61 ppm, J_{AB} = 11·7 Hz). The ^{13}C-NMR spectrum exhibits signals of eighteen carbon atoms. According to the off-resonance experiment, all the 27 hydrogen atoms are bound to carbon atoms and form four methine, seven methylene and three methyl groups. The ^{13}C-NMR chemical shifts show that oxynemorensine is a diester (carbonyls at 174·1 and 170·4 ppm) which does not contain any C=C bond. The proton at 5·46 ppm is directly coupled to the carbon at 75·3 ppm and can therefore be assigned to $H_{(7')}$. From its width it follows that the pyrrolizidine ring is exo-buckled[7].

Alkaline hydrolysis of oxynemorensine gave an acid which was identical with nemorensic acid (*VI*), found earlier[1] as a constituent of nemorensine (*I*). Furthermore by hydrolysis there was isolated a base whose melting point differed from that of platynecine (*VII*); the value of optical rotation was approximately the same. The ^{1}H-NMR spectrum in deuteriumoxide and that in deuteriochloroform differ from those of all the known isomers of 7′,9′-dihydroxynecines[6].

On the basis of the above-mentioned findings it is possible to interpret the NMR spectra of this alkaloid. In the ^{1}H-NMR spectrum, the signals of all the significant protons of the acidic moiety can be ascribed to $H_{(8)}$, $H_{(9)}$, $H_{(10)}$, $H_{(6)}$, and to one of the $H_{(4)}$. In the ^{13}C-NMR spectrum of oxynemorensine, the signals of two ester carbonyls (174·1 and 170·4 ppm), two quaternary carbon atoms bound to oxygen (85·6 and 82·8 ppm), two methylene carbon atoms (47·0 and 42·8 ppm), one aliphatic methine carbon atom (41·3 ppm), and three methyl carbon atoms (32·0, 19·0 and 13·6 ppm) are attributable to nemorensic acid (*VI*). Compared to nemorensine (*I*), the differences in the chemical shifts of these carbon atoms are small (Fig. 1). To the necine moiety there are attributable the signals of the carbon atoms at 73·5 ppm ($C_{(7')}$), 72·8 ppm ($C_{(9')}$) on the basis of the off-resonance multiplicity, the signal of the methine carbon at 36·5 ppm ($C_{(1')}$), the signals of the methylene carbons at 69·0, 60·2, 31·9, and 25·0 ppm and, finally, the signal of the methine carbon atom at 86·2 ppm which is assigned to $C_{(8')}$ by elimination. There remain to be interpreted the large downfield shifts of some signals. The ^{13}C-NMR data can be used for the verification of the oxygen atom balance. Nemorensic acid (*VI*) contains five oxygen atoms (two ester groups and an oxygen bridge of the tetrahydrofurane ring)

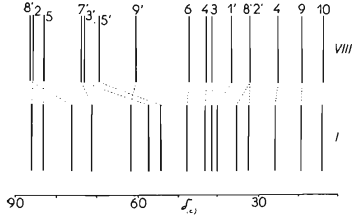


FIG. 1

A Comparison of ^{13}C-NMR Chemical Shifts of Oxynemorensine (*VIII*) and Nemorensine (*I*)

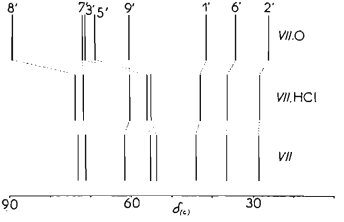
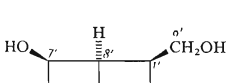


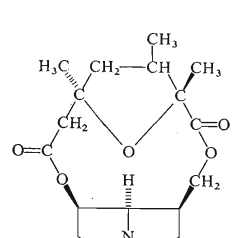
FIG. 2

A Comparison of ^{13}C-NMR Chemical Shifts of Platynecine (*VII*), Platynecine Hydrochloride (*VII*.HCl), and Platynecine-N-oxide (*VII*.O)

and it is attached to the primary alcohol and secondary alcohol necine group. In the ^{13}C-NMR spectrum, all the carbons of the mentioned function groups exhibit corresponding signals. Besides them there are, however, no other carbon atoms bound to oxygen found present. From this it is concluded that the sixth oxygen atom in the molecule of oxynemorensine is bound to nitrogen. The positive charge on this atom in N-oxides can account for the downfield shift of the signals of atoms in its vicinity. Even the base isolated from alkaline hydrolysis must be an N-oxide as results from a comparison of its ^{13}C-NMR spectrum with the spectra of platynecine (*VII*) and its hydrochloride (Fig. 2).



VII



VIII

The observed downfield shifts of the signals $C_{(8')}$, $C_{(3')}$, $C_{(5')}$ and the upfield shifts of the signals $C_{(1')}$, $C_{(2')}$, and $C_{(6')}$ take the same direction as those of the pair platynecine–platynecine . HCl but they are larger. The N-oxide hypothesis is also corroborated by the mass spectra of the alkaloid and the base. The cluster of the ions M−16, M−17, and M−18 (M−O, M−OH, and M−H_2O) is characteristic for N-oxides[8]. There is good agreement with the spectrum of nemorensine (*I*) except for the ions *m/z* 238, 211, and 183 (shift by one mass unit) which have a different origin with oxynemorensine. The mass spectrum of necine from hydrolysis of oxynemorensine does not contain a molecular ion (the ion with the highest *m/z* corresponds to M−16); it contains, however, all the ions described in the spectrum of the N-oxide of platynecine[9]. The melting point of necine isolated from the hydrolysis of oxynemorensine also corresponds to the value published[10] for the N-oxide of platynecine (*VII*). Reduction of oxynemorensine with zinc in acidic medium gives nemorensine (*I*) and thus confirmatory evidence has been provided that oxynemorensine has structure *VIII*.

The IR spectrum (chloroform) of the alkaloid SN-D exhibits a broad band of carbonyls at 1718 cm^{-1} and a band corresponding to the C=C bond at 1645 cm^{-1} but no band corresponding to hydroxyl groups. In the ultraviolet region of the

spectrum, the alkaloid SN-D absorbs at 219 nm (log ε 3·94, calculated to the molecular mass 350), which indicates[4] that probably an unsaturated acid is present in the molecule. The ^{1}H-NMR spectrum shows, however, that this alkaloid is not pure and even by repeatedly carried out chromatography a substance of satisfactory purity cannot be obtained.

Thin layer chromatography of the portion containing alkaloids from N-oxides showed the same qualitative composition as that of free alkaloids. It has already been found earlier[1,2] that even in the other varieties of *S. nemorensis*, the portion of alkaloids present in the plant in form of N-oxides is considerably higher than that of free alkaloids. The mixture of alkaloids from N-oxides was separated into two different polar fractions. The less polar fraction contained the alkaloids nemorensine (*I*), retroisosenine (*II*), and bulgarsenine (*III*), and was not separated further. The more polar portion gave the alkaloids oxynemorensine (*VIII*) and an impure alkaloid SN-D. In this portion the occurrence of N-oxides (oxynemorensine) is surprizing and indicates an imperfect reduction of the aqueous solution of N-oxides of alkaloids before extraction with chloroform.

To establish the relationship between the unsaturated acid *IV* and nemorensic acid (*VI*), an attempt was made to carry out the conversion of bulgarsenine (*III*) to nemorensine (*I*). Addition of hydrogen chloride to bulgarsenine (*III*) under catalysis with aluminium chloride and following alkalization led to closure of the tetrahydrofurane ring between $C_{(5)}$ and the oxygen of the hydroxyl group at $C_{(2)}$. This addition must have proceeded stereospecifically because the obtained substance was in all respects identical with nemorensine (*I*). Thus confirmatory evidence was provided that in all the chirality centres the absolute configuration of the unsaturated acid *IV* is the same as that of nemorensic acid (*VI*). From this point of view it appears possible that nemorensic acid (*VI*) and *cis*-nemorensic acid (*V*) will differ from each other by the absolute configuration at $C_{(5)}$.

EXPERIMENTAL

The melting points have been determined on a Kofler block. The UV spectra were measured in a Unicam SP-700 spectrophotometer in 95% ethanol, the IR spectra in a UR-20 apparatus (Zeiss Jena, GDR) in chloroform or in KBr pellets, the ^{1}H-NMR spectra in a Varian T-60 (CW-mode) and in a Jeol FX-60 (FT-mode) with tetramethylsilane as internal standard, and the ^{13}C-NMR spectra in a Jeol FX-60 (15·036 MHz, FT-mode). The alkaloids were dissolved in deuteriochloroform and the necines in deuteriumoxide (dioxane $\delta_{(C)} = 67{\cdot}4$ ppm as internal standard) at 20°C. The chemical shifts are expressed in δ values and have been calculated from digitally obtained differences of addresses ($\pm 0{\cdot}005$ and $\pm 0{\cdot}06$ ppm for ^{1}H and ^{13}C-NMR, respectively). The assignment of signals was based on the results of the off-resonance decoupling, selective decoupling, comparison with related compounds, and on general chemical shift considerations. The mass spectra were measured with a Varian MAT 311 spectrometer (70 eV, direct inlet at 120—160°C). High-resolution measurements (± 3 ppm) were carried out by the peak-matching technique with a perfluorokerosene standard. Thin layer chromatography on silica

gel G (Merck) was carried out using the solvent systems S_1 (benzene–ethyl acetate–diethylamine (7 : 2 : 1), detection with Dragendorff reagent) and S_2 (benzene–methanol–dioxane–acetic acid (45 : 4 : 4 : 4), detection with bromocresol green). The solutions of all the substances in organic solvents were dried with anhydrous sodium sulphate.

Isolation of Alkaloids

The roots of *S. nemorensis* L., var. *subdecurrens* GRISEB. (15 kg) were collected in August 1973 on the mountain Vitosha in Bulgaria. After drying at 30°C and grinding they were extracted with seven 30 litre portions of methanol. The methanolic extract was concentrated *in vacuo* to a volume of 2 l, the residue diluted with water 1 : 1, and acidified with a saturated solution of citric acid. The solution was gradually extracted 3times with a mixture of light petroleum–ether (1 : 1) and 3times with ether (à 400 ml). The extracts were combined and washed twice with a 10% solution of sodium bicarbonate. This solution was washed with ether, acidified with diluted sulphuric acid to Congo red and extracted with ether to afford 33·4 g of a crude mixture of acids.

The acidic aqueous layer after extraction with light petroleum and ether was made alkaline with concentrated ammonia to pH 10·5 and extracted 5times with 400 ml of chloroform. After drying and evaporation of chloroform the yield gave 94 g of a crude mixture of alkaloids.

The alkaline solution after extraction with chloroform was acidified with hydrochloric acid to Congo red under cooling, zinc powder (100 g) was added and the solution left standing overnight. After filtration, the solution was made alkaline with concentrated ammonia and extracted 5times with 400 ml of chloroform. After drying and evaporation of chloroform the yield gave 107·4 g of a crude mixture of alkaloids from N-oxides.

Purification and Separation of Acids

The crude mixture of acids (33·4 g) was extracted with a saturated solution of sodium hydrogen carbonate, the solution was filtered, washed 3times with ether, filtered over active charcoal, acidified with hydrochloric acid to Congo red, and extracted 5times with ether. After drying and distillation a yellow vitreous residue (6·95 g) was obtained. Crystallization from a mixture of ethyl acetate–light petroleum and following crystallization from ethyl acetate gave 1·3 g of a substance of m.p. 168—171°C, hR_F 38 (S_2) which on the basis of the mixed melting point and the IR spectrum was identical with the unsaturated acid *IV*, isolated[1] already earlier from this plant. The mother liquors after crystallizaton of the *IV* (5·5 g) were chromatographed on 500 g of silica gel (Merck, mesh smaller than 0·08 mm), saturated with 0·25M sulphuric acid (10% of the mass) in a column ∅ 5 × 60 cm, fractions amounting to 50 ml. The fractions 1—51 (chloroform–acetone, 9 : 1 up to 4 : 1) gave neutral substances (0·6 g) with traces of an acid of hR_F 80 (S_2), on the basis of thin layer chromatography identified as fumaric acid which was demonstrated in this plant earlier[1]. The fractions 55—72 (chloroform–acetone, 7 : 3) yielded a pure substance (1·95 g) of hR_F 53 (S_2), which crystallized after two months standing. Recrystallization from a mixture of ethyl acetate–light petroleum gave 164 mg of crystals of m.p. 100—104°C which on the basis of the IR and ^{1}H-NMR spectra were identified as *cis*-nemorensic acid (*V*). This compound had been isolated[2] as an amorphous product of the hydrolysis of bulgarsenine (*III*). The fractions 78—100 (chloroform–acetone, 1 : 1 up to acetone) (0·76 g) crystallized from the mixture ethyl acetate–light petroleum to give the unsaturated acid *IV*, m.p. 169 to 171°C, hR_F 38 (S_2), which was identified on the basis of the mixed melting point and the IR spectrum. The fractions 52—54 (0·3 g) and 73—77 (0·47 g) yielded an amorphous mixture of substances of hR_F 61 and 53, and 53 and 45 (S_2), respectively. The substance of hR_F 45 (S_2) corresponded to nemorensic acid (*VI*), the mixture could, however, not be separated further by chromatography.

Conversion of the Acid *IV* to *cis*-Nemorensic Acid (*V*)

A mixture of the unsaturated acid *IV* (700 mg), KOH (800 mg) and ethanol (20 ml) was refluxed for 4 hours, evaporated in vacuum, the residue washed twice with hot chloroform, dissolved in water, acidified with 15% sulphuric acid, and extracted 5times with ether. After evaporation of ether, a residue (580 mg) was obtained which could not be brought to crystallization. Thin layer chromatography revealed the presence of *cis*-nemorensic acid (*V*), (hR_F 53), the main portion was formed by the unreacted acid *IV* (hR_F 38). On chromatography of the mixture of acids on silica gel saturated with 0·25M sulphuric acid (10% of the mass) by application of the same technique as that used on separation of the acidic extract, both acids were separated to give the acid *IV* (350 mg), m.p. 168—170°C, and an oily *cis*-nemorensic acid (*V*) (53 mg), the identity of which was confirmed by comparison of its IR and ^{1}H-NMR spectra with those of the authentic sample.

Purification and Separation of the Crude Mixture of Alkaloids

The crude mixture of alkaloids (94 g) was extracted with 1000 ml of 3% sulphuric acid, the insoluble portion was filtered off, the filtrate washed 3times with ether and 2times with benzene, made alkaline with concentrated ammonia, and extracted 5times with chloroform. After drying and evaporation of chloroform, a repurified mixture of alkaloids (23·7 g) was obtained.

The repurified mixture of alkaloids (11·2 g) was chromatographed on aluminium oxide (380 g, activity II (Reanal)) in a column ⌀ 4·5 cm, fractions amounting to 17 ml. Before separation, the column with the applied extract was washed with 300 ml of benzene. The residue gave 3 g of Dragendorff-negative substances. The fractions 1—98 (benzene–ethanol, 99·5 : 0·5 up to 99 : 1) yielded again only Dragendorff-negative substances (17 mg). The fractions 99—118 (benzene–ethanol, 99 : 1) gave a mixture (1·183 g) of the alkaloids retroisosenine (*II*, hR_F 61, S_1) and bulgarsenine (*III*, hR_F 55, S_1) (portion *A*), the fractions 119—320 (benzene–ethanol, 99 : 1 up to 98 : 2) a mixture (1·03 g) of retroisosenine (*II*), bulgarsenine (*III*) and nemorensine (*I*, hR_F 42, S_1), and of a substance of hR_F 48 (portion *B*). The mixture of bulgarsenine (*III*), nemorensine (*I*), and of the substances of hR_F 32 and 24 (0·72 g) was obtained from the fractions 321—535 (benzene–ethanol, 98 : 2 up to 1 : 1) (portion *C*). The fractions 536—590 (benzene–ethanol, 1 : 1) yielded 0·43 g of a substance of hR_F 24 (S_1) with traces of nemorensine (*I*) (portion *D*). Washing of the column with two 250 ml portions of ethanol gave in addition 0·73 and 0·53 g of a mixture of substances of hR_F 32, 24 and 0 (portion *E*). These mixtures were combined with analogous fractions from chromatography of the second portion of the mixture of alkaloids (11·2 g) and subjected to further separation.

The portion *A* (1·1 g) was chromatographed on 110 g of silica gel (Merck, 70—325 mesh) in a column ⌀ 3·5 cm. The solvent system was a mixture of benzene–ethyl acetate–diethyl amine (85 : 10 : 5), fractions amounting to 5 ml. Pure retroisosenine (*II*, 0·3 g), m.p. 126—127°C, $[\alpha]_{24}^{D}$ +117° ± 2° (*c* 0·44 in chloroform) was obtained from the fractions 110—135, bulgarsenine (*III*, 0·31 g), m.p. 114—115°C, $[\alpha]_D^{24}$ −56° ± 2° (*c* 0·55 in chloroform) from the fractions 136 to 182, and nemorensine (*I*, 0·05 g), m.p. 132—133°C, $[\alpha]_D^{24}$ −57° ± 2° (*c* 0·38 in chloroform) from the fractions 183—250. ^{13}C-NMR nemorensine (*I*): 174·8 s, 171·5 s, 85·9 s, 83·0s, 75·9d, 70·9 d, 61·5 t, 57·0 t, 54·1 t, 47·6 t, 43·1 t, 41·5 d, 40·0 d, 35·3 t, 32·4 q, 25·7 t, 19·4 q, 14·0 q.

The combined portions *C*, *D* and the first part of the ethanolic extract *E* (4·3 g) were chromatographed on aluminium oxide (130 g, activity II (Reanal), column ⌀ 3 cm), fractions amounting to 13 ml. Prior to chromatographic separation, the column with the applied mixture of alkaloids was washed with a mixture of benzene–ethanol (95 : 5) (600 ml). The yield gave 0·2 g of Dragendorff-negative substances. The fractions 11—63 (benzene–ethanol, 95 : 5 up to 1 : 1) gave a substance of hR_F 24 (S_1) with traces of a substance of hR_F 32 (S_1). The residue (0·8 g) was dissolved

in ethanol, filtered and, after addition of ethanolic hydrogen chloride, the solvent was evaporated *in vacuo*. Since the residue of hydrochloride could not be brought to crystallization, it was dissolved in water, filtered over active charcoal, made alkaline with ammonia and extracted 3times with chloroform. After drying and evaporation, a yellow vitreous substance SN-D (250 mg) (hR_F 24, S_1) was obtained. The ^{1}H-NMR spectrum showed that the substance was not pure and so far it could not be purified chromatographically; it was also not possible to prepare the crystalline picrate. The fractions 1—10 yielded a mixture containing the alkaloid SN-D and bulgarsenine (*III*), retroisosenine (*II*), and a substance of hR_F 32 (0·4 g). Washing of the column with ethanol (500 ml) gave 2·4 g of a mixture of substances of hR_F 24 and 0 (S_1).

The second part of the portion *E* was combined with the ethanolic portion obtained from the preceding chromatography (3 g) and chromatographed on silica gel (300 g) (Merck, 70—325 mesh) in a column ∅ 4·5 cm, solvent system S_1, fractions amounting to 15 ml. The fractions 104 to 124 yielded 531 mg of a residue of hR_F 32 (S_1) which was dissolved in 0·1M-HCl, filtered over active charcoal, made alkaline with ammonia, and extracted 5times with chloroform. After drying and evaporation, a residue (100 mg) was obtained which crystallized from ethyl acetate to afford 55 mg of oxynemorensine (*VIII*) (alkaloid SN-E), m.p. 160—163°C, $[\alpha]_D^{20}$ —35° ± 2° (*c* 1·0 in chloroform). The substance did not absorb in the UV spectrum and on attempted acetylation it polymerized. ^{13}C-NMR oxynemorensine (*VIII*): 174·1 s, 170·4 s, 86·2 d, 85·6 s, 82·8 s, 73·5 d, 72·8 t, 69·0 t, 60·2 t, 47·0 t, 42·8 t, 41·3 d, 36·5 d, 32·0 q, 31·9 t, 25·0 t, 19·0 q, 13·6 q. Mass spectrum of oxynemorensine (*VIII*): *m/z* (% relative intensity, elemental composition): 353 (1·5, $C_{18}H_{27}NO_6$, M^+), 337 (4·5, $C_{18}H_{27}NO_5$), 336 (1·2, $C_{18}H_{26}NO_5$), 335 (0·7, $C_{18}H_{25}NO_5$), 322 (0·8, $C_{17}H_{24}NO_5$), 237 (6·6, $C_{13}H_{19}NO_3$), 222 (2·9, $C_{13}H_{20}NO_2$), 210 (6·1, $C_{11}H_{16}NO_3$), 183 (7·5, $C_{10}H_{17}NO_2$), 138 (16·8, $C_8H_{12}NO$), 123 (38·9, $C_8H_{13}N$), 122 (34·7, $C_8H_{12}N$), 119 (28·4, C_8H_9N), 82 (100, C_5H_8N). The fractions 211—220 gave 370 mg of a residue which on crystallization from acetone yielded 45 mg of a substance of m.p. 203 to 205°C, which was identified as diethylamine hydrochloride (MS, ^{1}H-NMR).

Purification and Isolation of Alkaloids from N-Oxides

The crude mixture of alkaloids from N-oxides (98·3 g) was dissolved in the heat in 100 ml of ethyl acetate. After addition of ether (1 000 ml) the precipitate was collected by sucking. The ethereal solution was evaporated and the residue processed in the same manner. Yield 63·6 g of a mixture of alkaloids soluble in a mixture of ethyl acetate–ether and 32 g of alkaloids insoluble in the mixture. The first portion contained (according to thin layer chromatography) the alkaloids retroisosenine (*II*), bulgarsenine (*III*), and nemorensine (*I*) with traces of the alkaloid SN-D, and it was not separated further. The second (more polar) portion contained particularly the alkaloids SN-D, oxynemorensine (SN-E, *VIII*), traces of nemorensine (*I*), and a substance of hR_F 0. This portion was separated chromatographically on a column of aluminium oxide in a similar manner as that described above; yield 510 mg of pure oxynemorensine (*VIII*) and 120 mg of the amorphous alkaloid SN-D which according to the ^{1}H-NMR spectrum contained impurities.

Alkaline Hydrolysis of Oxynemorensine (*VIII*)

The mixture of oxynemorensine (*VIII*, 300 mg), potassium hydroxide (300 mg) and methanol (12 ml) was refluxed for 4 hours and evaporated in vacuum. The residue was extracted 5times with hot chloroform. After evaporation of the solvent, a crude base (94 mg) was obtained which was dissolved in ethanol, and after addition of the ethanolic hydrogen chloride the solvent was removed by evaporation. The hydrochloride could not be brought to crystallization and

therefore it was dissolved in water, filtered through a column of Amberlit IRA-401 in the OH^- cycle and the filtrate evaporated *in vacuo*. The yield gave 55 mg of a residue which crystallized from acetone to give 30 mg of necine, m.p. 215—218°C, $[\alpha]_D^{20}$ —52° ± 2° (*c* 0·56 in ethanol). The N-oxide of platynecine (*VII*) was reported[10] to have m.p. 217—218°C. The ^{13}C-NMR of the N-oxide of platynecine: 89·2 d, 71·9 d, 71·2 t, 68·7 t, 60·4 t, 41·3 d, 34·1 t, 25·9 t. The ^{13}C--NMR of platynecine (*VII*): 73·0 d, 71·1 d, 61·5 t, 55·2 t, 53·7 t, 43·9 d, 36·4 t, 28·5 t. The ^{13}C--NMR of platynecine hydrochloride: 73·7 d, 71·7 d, 60·3 t, 56·0 t, 55·0 t, 42·8 d, 36·2 t, 28·3 t. Mass spectrum of N-oxide of platynecine: *m/z* (% relative intensity, elemental composition): 157 (5·7, $C_8H_{15}NO_2$), 113 (16·7, $C_6H_{11}NO$), 106 (8·0, C_7H_8N), 96 (6·3, $C_6H_{10}N$), 82 (100, C_5H_8N).

The potassium salt of the acid after extraction with chloroform was dissolved in water, acidified with 15% of sulphuric acid and extracted 5times with ether. After evaporation of the extract, 165 mg of the crude acid were obtained, dissolved in acetone, the solution filtered, evaporated and crystallized from ethyl acetate. Yield 120 mg of needles, m.p. 175—178°C, $[\alpha]_D^{20}$ +94 ± 2° (*c* 0·64 in ethanol). On the basis of the mixed melting point and the IR spectrum, the substance was identified as nemorensic acid (*VI*).

Reduction of Oxynemorensine (*VIII*) to Nemorensine (*I*)

To a solution of oxynemorensine (*VIII*, 100 mg) in 3 ml of 1M-H_2SO_4, several crystals of cupric sulphate and 100 mg of zinc powder have been added. The mixture was mixed at laboratory temperature for 3 hours. The undissolved zinc was filtered off, the filtrate made alkaline with ammonia up to dissolution of the transiently formed precipitate and extracted 5times with chloroform. After drying and evaporation of chloroform, a residue (65 mg) was obtained which crystallized from ethyl acetate to afford 25 mg of a substance of m.p. 132—134°C, $[\alpha]_D^{24}$ —59° ± 2° (*c* 0·46 in chloroform), whose identity with nemorensine (*I*) was confirmed by mixed melting point and IR spectra.

Conversion of Bulgarsenine (*III*) to Nemorensine (*I*)

Bulgarsenine tartrate (*III*) $(CHOH)_2.(COOH)_2$ (650 mg) was suspended in 20 ml of acetone, and acetic acid was added up to the dissolution of the suspension (10 ml). After addition of 200 mg of anhydrous aluminium chloride, the mixture was saturated at 0°C with dry hydrogen chloride and left standing for 3 days at laboratory temperature. Then decomposed by addition of water (1 ml) and evaporated in vacuum. The residue was dissolved in 30 ml of water, the solution was washed 2times with ether, made alkaline with ammonia and extracted 5times with chloroform. After drying and evaporation of chloroform, the yield gave 265 mg of an oily residue which was extracted with ethyl acetate, filtered and evaporated. The residue was extracted with 0·1M-HCl, the extract washed 2times with ether, made alkaline with ammonia and extracted 3times with chloroform. After drying and evaporation, an amorphous residue (145 mg) was obtained which was converted into a picrate (140 mg), m.p. 226—228°C (ethanol); on the basis of the melting point and the IR spectrum it was identical with the picrate of authentic nemorensine (*I*). The picrate was converted to a free base in the usual manner to afford 52 mg of crystals of m.p. 131—133°C (ethyl acetate), $[\alpha]_D^{24}$ —60° ± 2° (*c* 0·98 in chloroform). The identity of the isolated substance with nemorensine (*I*) was confirmed by determination of the mixed melting point and comparison of the IR and ^{1}H-NMR spectra.

Note added on June 15th, 1979: E. Röder and H. Wiedenfeld and H. Wiedenfeld and E. Röder (Phytochemistry *16*, 1462 (1977); *18*, 1083 (1979)) isolated the alkaloids fuchsisenecionine and

senecionine from *S. nemorensis* L., ssp. *fuchsii* GMELIN (plant material from various parts of Europe). They did not succeed to isolate the alkaloids nemorensine, retroisosenine, and bulgarsenine.

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