PYRROLIZIDINE ALKALOIDS. XX.*

NEMORENSINE, AN ALKALOID FROM *Senecio nemorensis* L.

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In this paper, the isolation of the alkaloid nemorensine from *Senecio nemorensis* L., var. *subdecurrens* GRISEB., *S. nemorensis* L., ssp. *Jacquinianus* (RCHB.) DURAND and *S. nemorensis* L., ssp. *fuchsii*, var. *nova* (ZLATNÍK) has been described. On the basis of some chemical reactions and the interpretation of the PMR and mass spectra, the structure *V* has been assigned to nemorensine.

While carrying out a systematic investigation of pyrrolizidine alkaloids, we have isolated the alkaloids contained in three different varieties of *Senecio nemorensis* L. The isolation of the alkaloid fuchsisenecionine and the base $C_9H_{15}NO_2$ from *S. fuchsii* (probably *S. nemorensis* L., ssp. *fuchsii*) has already been reported earlier by Müller[1]. These alkaloids have, however, not been described in more detail and no mention has been made of them in recent literature.

By application of the conventional method of isolation, a so far undescribed alkaloid nemorensine has been obtained from the three plants under investigation. The spectrum of alkaloids contained in them is practically the same. In the Czechoslovak plants *S. nemorensis* L., ssp. *Jacquinianus* and *S. nemorensis* L., ssp. *fuchsii*, the content of alkaloids is, however, much lower than that in the Bulgarian plant *S. nemorensis* L., var. *subdecurrens* GRISEB. Since the quantity of the available plant material was very small, only nemorensine could be isolated from the mixture in pure state.

The acidic ether extract of *S. nemorensis* L., var. *subdecurrens* GRISEB., gave dicarboxylic acid $C_{10}H_{16}O_5$. Its UV spectrum (λ_{max} 220 nm, $\log \varepsilon$ 4·12) shows a double bond conjugated with a carboxyl and its IR spectrum (nujol) two carboxyl bands at 1730 and 1687 cm^{-1}, a band at 1642 cm^{-1}, which is attributable to a double bond, and a very sharp hydroxyl band at 3450 cm^{-1}. The structure of this dicarboxylic acid can be derived from its PMR spectrum (measured in a mixture of hexadeuteriobenzene and hexadeuteriodimethyl sulphoxide). This spectrum exhibits a singlet

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of a tertiary methyl at 1·44 p.p.m., a doublet (J = 6·8 Hz) of the secondary methyl at 1·02 p.p.m., a doublet (J = 1·3 Hz) of the olefinic methyl at 1·68 p.p.m., a multiplet of the methine proton of the secondary methyl at 2·28 p.p.m. (splitting 6·8, 10·0, and 4·0 Hz), two one-proton quartets at 2·63 p.p.m. (J = 12·0 and 10·0 Hz) and at 3·12 p.p.m, (J = 12·0 and 4·0 Hz), and a multiplet of the olefinic proton at 5·84 p.p.m. The remaining three protons, whose signals have not been observed in the spectrum, are ascribed to two carboxyl and to one hydroxyl protons. The empirical formula indicates three unsaturations which are attributable to two C=O groups of carboxyl, the third unsaturation is due to a trisubstituted double bond whose substituents are the methyl, the carboxyl, and the aliphatic chain. The magnitude of the coupling of the olefinic proton to the methyl (1·3 Hz) excludes the possibility of a geminal arrangement. The chemical shift of the signal of the olefinic methyl indicates that this group is in *trans* configuration to the carboxyl. The olefinic proton shows, besides the already mentioned 1·3 Hz coupling to the methyl, still other (double resonance proved) couplings to the protons at 2·63 and 3·12 p.p.m. The magnitudes of these couplings correspond to allylic interactions. The 12·0 Hz coupling between the last two protons can be considered as a geminal coupling. The magnitudes of the couplings of these protons with the secondary methyl methine proton correspond to the vicinal couplings; the proton at 2·63 p.p.m. is situated in antiperiplanar position to the methine proton. Since this methine proton has only the three above mentioned couplings, the next atom in the chain does not carry any hydrogens. The three remaining groups are then attached to it, namely the tertiary methyl, the hydroxyl, and the carboxyl. The chemical shift of the tertiary methyl is in agreement with these conclusions. Consequently, the structure of dicarboxylic acid is expressed by the formula *I*. The mass spectrum can be rationalized on the basis of this structure.

The mass spectrum of the alkaloid nemorensine displays a molecular ion at m/e 337 ($C_{18}H_{27}NO_5$, according to high resolution). Other prominent peaks appear at m/e 184, 138, 123, 122 and 82 (base peak). Evidence for the classification of nemorensine to pyrrolizidine alkaloids with a saturated base[2,3] is provided by the presence of ions at m/e 123, 122, and 82. The large band in the 1735–1705 cm^{-1} region of the infrared spectrum (chloroform) was assigned to the carbonyls of the ester groups. The shape of the spectrum in the region above 3000 cm^{-1} indicates the absence of hydroxyl groups. Nemorensine does not absorb in the UV region from which follows[4] that neither the acidic nor the basic moiety of the molecule has a double bond. The PMR spectrum (deuteriochloroform) shows singlets of two tertiary methyls at 1·27 and 1·36 p.p.m., a doublet of the secondary methyl (J = 6·6 Hz) at 0·98 p.p.m., a three proton multiplet in the region from 3·2 to 3·5 p.p.m., two one-proton quartets at 4·15 p.p.m. (J = 12·5 and 0·9 Hz) and 4·53 p.p.m. (J = 12·5 and 3·7 Hz), and a one-proton multiplet at 5·03 p.p.m. Assuming the presence of a saturated pyrrolizidine base *II*, we attribute the multiplet at 5·03 p.p.m. to the signal of the proton $H_{(7')}$. The two above mentioned quartets might be assigned to the pro-

tons at $C_{(9')}$ on the basis of $J = J_{gem} = 12{\cdot}5$ Hz. The absence of other signals above 4·0 p.p.m. is consistent with the assumed saturation of the basic component. In the rest of the spectrum, tickling experiments revealed the AB system of two protons at 2·23 and 2·59 p.p.m. ($J = 12{\cdot}5$ Hz) and two protons connected by an 11·5 Hz coupling; the one proton appearing as a triplet at 1·59 p.p.m. and the second as a quartet ($J = 7{\cdot}5$ and 11·5 Hz) at 2·74 p.p.m. The signals of these protons together with the signals of the two tertiary methyls and the secondary methyl are, therefore, attributable to the acidic moiety of the molecule. Addition of several drops of tetradeuterioacetic acid to the measured sample causes a shift of some signals; there appears a new quartet at 4·32 p.p.m. ($J = 3{\cdot}2$ and 7·0 Hz) and the structure of the triplet at 1·65 p.p.m. becomes more resolved. Double resonance experiments showed that the proton at 4·32 p.p.m. has a 3·2 Hz coupling to a multiplet at 5·43 p.p.m. which has already been assigned to $H_{(7')}$. The great downfield shift after acidification (*c.* 1·0 p.p.m., this signal originally appeared in the region between 3·2–3·5 p.p.m.) shows that the signal at 4·32 p.p.m. is attributable to the proton $H_{(8')}$. From a comparison of the magnitude of the vicinal couplings $J_{7',8'}$ and $J_{1',8'}$ with analogous values obtained from known pyrrolizidine alkaloids containing saturated bases and with those of necines (Table I), it is concluded that the basic moiety of nemorensine is platynecine or its diastereoisomer. The width of the multiplet $H_{(7')}$ shows that the two rings of the pyrrolizidine system are *exo*-buckled[5]. The unusually low values of the two couplings $J_{1',9'u}$ and $J_{1',9'd}$ can be explained by a forced conformation of the macroester ring.

Alkaline hydrolysis of nemorensine afforded nemorensic acid $C_{10}H_{16}O_5$. This acid does not absorb in the UV region and its IR spectrum (nujol) does not exhibit a band in the region of the hydroxyl groups and in that between 1650–1600 cm^{-1}, which evidences its saturation. Likewise, its PMR spectrum (in hexadeuteriodimethyl sulphoxide) does not display any signals in the region of olefinic protons. There were observed the signals of two tertiary methyls at 1·32 and 1·42 p.p.m., a doublet ($J = 6{\cdot}5$ Hz) of a secondary methyl at 1·13 p.p.m., and a broad band of two carboxyl protons at 10·0 p.p.m. The signals of the remaining five protons were present in the region between 1·5 and 2·9 p.p.m. Their nature can be better explained by interpretation of the PMR spectrum of the dimethyl ester of nemorensic acid (Table II) which was prepared by reaction with diazomethane. The methine proton of the secondary methyl is coupled to the two protons whose 12·5 Hz coupling might be considered as a geminal coupling. These two protons have no other couplings, which leads to the formulation of a partial structure $CH_3—\underset{\blacksquare}{\overset{|}{C}}H—CH_2—\blacksquare$.

The presence of a pure AB system in the PMR spectrum indicates two protons with two quaternary carbon atoms in its vicinity. The chemical shifts of the protons of the AB system (2·23 and 2·59 p.p.m.) are consistent with the aliphatic or epoxide nature

TABLE I

A Comparison of the Values of the Coupling Constants J of Nemorensine and Pyrrolizidine Alkaloids with Saturated Necines

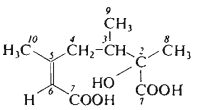
Compound	$J_{7',8'}$	$\sum J_{7'}$	$J_{1',8'}$	$J_{1',9'u}$	$J_{1',9'd}$	$J_{9'u,9'd}$
Platynecine[3]	3·0	8·4	8·2	$1/2 = 5·5$		—
Dihydroxyheliotridane[3]	7·1	20·3	7·4	7·1	7·6	10·8
Hastanecine[3]	—	12·0	—	6·5	4·6	11·0
Turneforcidine[3]	4·7	14·1	—	—	—	—
Platyphylline[3]	4·8	14·1	7·0	2·1	9·0	11·6
Platyphylline[5]	4·9	14·3	5·0	2·1	8·9	11·5
Neoplatyphylline[3]	5·8	17·4	6·5	2·3	9·7	11·1
Hastacine[3]	9·1	24·2	—	1·6	10·8	10·8
Retusine[3]	9·3	23·9	7·8	10·7	3·2	10·7
Rosmarinine	3·2	10·0	8·0	1·0	5·5	13·0
Nemorensine	3·2	7·0	7·0	0·9	3·7	12·5

TABLE II

PMR Data of Acid Protons

Proton	*I*	*III*	*IV*	*V*
$H_{(3)}$	2·28 m (6·8, 10·0, 4·1)	2·0—2·8	2·64 m (6·8, 7·0, 10·6)	—
$G_{(4d)}$	3·12 q (12·0, 4·0)	2·11 q (12·0, 6·0)	2·41 q (12·5, 7·0)	2·74 q (11·5, 7·5)
$H_{(4u)}$	2·63 q (10·0, 12·0)	1·69 t (12·0)	1·56 q (12·5, 10·6)	1·59 t (11·5)
$H_{(6)}$	5·84 m (1·3)	2·0—2·8	2·53, 2·68 $J_{AB} = 12·5$	2·23, 2·59 $J_{AB} = 12·5$
$H_{(8)}$	1·44 s	1·42 s	1·43 s	1·36 s
$H_{(9)}$	1·02 d (6·8)	1·13 d (6·5)	1·08 d (6·8)	0·98 d (6·6)
$H_{(10)}$	1·68 d (1·3)	1·32 s	1·38 s	1·27 s
COOH	—	10·0 s	—	—
$COOCH_3$	—	—	3·66 s 3·73 s	—

of these protons. The coupling constant $J = 12{\cdot}5$ Hz is, however, too large for both the vicinal and the geminal couplings between epoxide protons[6,7]. They most probably represent two aliphatic protons, attached either to two adjacent carbon atoms and oriented *trans*-diaxially, or situated on the same carbon atom. The first possibility can, however, be excluded because it would require the addition of four quaternary carbon atoms to the fragment CH—CH but there are available only two —COOH groups and only one quaternary carbon atom. Therefore, the above mentioned AB system can only be accounted for by an isolated methylene group. Thus the nature of all the hydrogen atoms is explained. The nature of two quaternary carbon atoms and one oxygen remains still to be defined. This oxygen atom might be either a constituent of another carbonyl or of an oxygen bridge. The presence of two tertiary methyls in the molecule of nemorensic acid indicates that in the case of the carbonyl both of them are attached to the same carbon atom. The second alternative would be the linkage of two quaternary carbon atoms (each of them carrying one methyl) with an oxygen bridge. These atoms have to be located in such a manner that the methylene group and the above mentioned fragment form isolated systems, which leads to the formation of a five-membered ring. The fragmentation pattern of nemorensic acid in the electron impact and, particularly, the presence of a stable ion M—COOH and the formation of eight-carbon hydrocarbon fragments favour the second alternative. In view of the skeletons of the so far known necic acids, it is possible to formulate nemorensic acid as *III*. A comparison of the structure *III* with that of the acid *I* indicates a possible relationship of these two acids. It is, however, difficult to say whether the acid *I* is a precursor of nemorensic acid or a product of the metabolic degradation of nemorensine.



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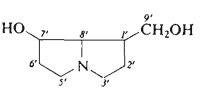
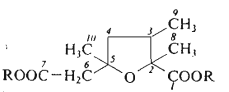
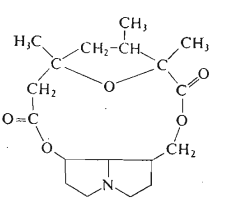


II



III, R = H
IV, R = CH_3



V

Even though the basic portion (it might be either platynecine or its diastereoisomer (*II*)) of nemorensine could not be obtained in pure state, the structure *V* can be ascribed to this alkaloid on the basis of the above mentioned facts.

EXPERIMENTAL

The melting points were determined on a Kofler block and are not corrected. The ultraviolet spectra were measured on a Unicam SP-700 instrument in 95% ethanol, the infrared spectra on an Infrascan H-900 in chloroform or nujol, the mass spectra on a G.E./A.E.I. MS-9 and a Varian MAT 311 instrument, and the CD spectra on a Roussel-Jouan dichrograph (model 185) in ethanol. The PMR spectra were measured on a Varian HA-100 with tetramethylsilane or hexamethyldisiloxane (HMDS) as internal standard, and the chemical shifts are expressed in δ-values ($\delta_{HMDS} = 0{\cdot}05$ p.p.m.). Thin-layer chromatography was performed on silica gel G (Merck) (solvent system benzene–ethyl acetate–diethylamine, 7 : 2 : 1, detection with Dragendorff reagent). Column chromatography was carried out on aluminium oxide (activity II, Reanal). The solutions of all the substances in organic solvents were dried over anhydrous sodium sulphate.

Isolation of Alkaloids

The dried and ground plant was continuously extracted with methanol. The extract was concentrated *in vacuo* to a syrupous consistence, diluted with water 1 : 1, acidified with a saturated solution of citric acid, and filtered. The filtrate was washed three times with light petroleum and extracted three times with ether (acidic ether extract). The aqueous layer was made alkaline with ammonia to pH 10·5 and extracted 5 times with chloroform. The alkaline chloroform extract was dried and the solvent removed by distillation to give a raw mixture of alkaloids. The aqueous layer was acidified with hydrochloric acid (1 : 1) to Congo-red, zinc dust was added (c. 1/50 of the plant weight), the mixture left standing for 2 days at laboratory temperature, and filtered. The filtrate was made alkaline with ammonia under cooling to pH 10·5, and extracted 5 times with chloroform. After drying and evaporation of the solvent, the chloroform extract gave a raw mixture of alkaloids from N-oxides. For purification, the crude mixtures of alkaloids were dissolved in chloroform, extracted with 0·5M sulphuric acid, filtered over active charcoal, made alkaline with ammonia and extracted with chloroform. After drying and evaporation of the solvent, the chloroform extract gave a purified mixture of alkaloids.

S. nemorensis L., var. *subdecurrens* GRISEB.

The dry and ground plant (1 815 g), collected in August 1960 at Rila horo (Bulgaria) gave a crude mixture of alkaloids (12·4 g), and a crude mixture of alkaloids from N-oxides (22·4 g). Thin-layer chromatography showed that the components of these two portions were the same (alkaloids SN-A hR_F 61, SN-B hR_F 55, SN-C hR_F 42, SN-D hR_F 24, and trace quantities of substances of hR_F 36 and 15) and, therefore, they were combined. Further purification yielded 7·8 g of a mixture of alkaloids. A separation of this mixture by column chromatography on aluminium oxide with the use of an eluotropic solvent scale[8] could not be achieved. After chromatography, this unseparated mixture (6·0 g) was rechromatographed on aluminium oxide (180 g, tube 35 mm i.d.), collection of 11 ml fractions. The fractions 1—39 (benzene–ethanol, 99 : 1) gave a Dragendorff-negative residue (0·75 g) which was not worked up further. The fractions 40—48 (benzene–ethanol, 98 : 2) yielded 1·3 g of a mixture of alkaloids SN-A, SN-B, and SN-C. Crystallization from cyclohexane afforded 160 mg of the alkaloid SN-C (nemorensine), m.p. 132—134°C (cyclo-

hexane), $[\alpha]_D^{24}$ $-58° \pm 2°$ (*c* 2·35 in chloroform), CD: 224 nm ($\Delta\varepsilon = +0{\cdot}24$). The UV spectrum of this substance does not show any absorption band. Mass spectrum: observed (calculated, composition): 337·1889 (337·1889, $C_{18}H_{27}NO_5$); 184·0971 (184·0974, $C_9H_{14}NO_3$); 138·0914 (138·0919, $C_8H_{12}NO$); 123·1041 (123·1048, $C_8H_{13}N$); 122·0966 (122·0970, $C_8H_{12}N$); 82·0656 (82·0657, C_5H_8N).

On an attempt to acetylate nemorensine in a mixture of acetic anhydride–pyridine, the unreacted starting material was recovered. The fractions 49—52 (benzene–ethanol 97 : 3) gave 0·59 g of a non-crystalline mixture of alkaloids SN-A, SN-B, and SN-C (portion 2), the fractions 53—67 (benzene–ethanol, 97 : 3) 0·36 g of a non-crystalline mixture of alkaloids SN-C and SN-D with traces of SN-A and SN-B (portion 3) and the fractions 68—113 (benzene–ethanol, 95 : 5 to 90 : 10) 0·30 g of a non-crystalline mixture of alkaloids SN-C, SN-D, and a substance of hR_F 15 (portion 4). The individual portions were then subjected to further separation.

The mother liquors after nemorensine (1·13 g, portion 1) were chromatographed on aluminium oxide, column 20 mm i.d., collection of 14 ml fractions. On the whole, 107 fractions were collected. The fractions 11—24 (benzene–chloroform, 90 : 10) gave 110 mg of nemorensine, m.p. 131—134°C. The other fractions contained an unseparated mixture of the alkaloids SN-A, SN-B, and nemorensine.

The portion 2 (0·59 g) was chromatographed on 55 g of silica gel (70—110 μ, Lachema, shaken with 2 ml of concentrated ammonia, column 25 mm i.d.), collection of 13 ml fractions. The fractions 71—95 (chloroform–ethanol, 99 : 1 to 90 : 10) gave 105 mg of a residue containing the alkaloid SN-A and traces of the alkaloid SN-B. The non-crystallizing mixture afforded a picrate which after recrystallization from ethanol gave 75 mg of yellow needles, m.p. 194—198°C. After conversion of the picrate into a free base, a glassy substance of hR_F 61 (SN-A) was obtained which according to the NMR spectrum was not pure. On further standing, the substance polymerizes. By washing the column with ethanol, a non-crystallizing residue (300 mg) was obtained which was converted to a picrate. Recrystallization of the picrate from ethanol gave 90 mg of yellow needles, m.p. 212—214°C, whose mixed melting point and IR spectrum were identical with the picrate of nemorensine.

The portion 3 (360 mg) was chromatographed on aluminium oxide (10 g, column 12 mm i.d.), collection of 10 ml fractions. Fraction 7 (benzene–ethanol, 97 : 3) gave a residue from which a picrate was prepared (15 mg), m.p. 211—214°C. On the basis of the IR spectrum and the mixed melting point, it is identical with the picrate of nemorensine. The fractions 8—9 (benzene–ethanol, 97 : 3) yielded a mixture of the alkaloids SN-C and SN-D and the fractions 10—42 (benzene–ethanol, 97 : 3 to 90 : 10) a residue which did not crystallize and did not yield a crystalline picrate. Thin-layer chromatography (hR_F 24) showed that it was the alkaloid SN-D. Acetylation of this substance with acetic anhydride in pyridine gave, however, a mixture of three substances of hR_F 63, 54, and 43.

The portion 4 (300 mg) was subjected to column chromatography (10 g of aluminium oxide), column 12 mm i.d.), but a pure substance could not be obtained.

S. nemorensis L., ssp. *Jacquinianus* (RCHB.) DURAND

The dry and ground plant (96 g), collected in July 1967 at the Finsterl cottage in Jeseníky, gave a crude mixture of alkaloids (0·19 g) and a crude mixture of alkaloids from N-oxides (0·18 g). Thin-layer chromatography showed that these two portions were identical. They were combined and purified to afford a mixture of alkaloids (73 mg) which was chromatographed on aluminium oxide (5 g, column 8 mm i.d.), collection of 10 ml fractions. The fractions 1—4 (benzene–ethanol, 99 : 1) gave a residue containing an alkaloid of hR_F 61 and traces of an alkaloid of hR_F 55

(perhaps SN-B). The picrate prepared from this portion was recrystallized from ethanol (2 mg), m.p. 195—198°C. Its mixed melting point and IR spectrum were identical with the picrate of the alkaloid SN-A from *S. nemorensis* L. Thin-layer chromatography of the fractions 5—7 (benzene–ethanol, 99 : 1) showed therein the presence of nemorensine (hR_F 42) with traces of a substance of hR_F 55 (perhaps SN-B). After recrystallization from ethanol, the picrate prepared from this portion gave yellow needles (10 mg), m.p. 227—229°C, which according to the IR spectrum and mixed melting point were identical with those of the picrate of nemorensine. Thin-layer chromatography of the fractions 10—22 (benzene–ethanol, 97 : 3 to 90 : 10) yielded a residue which contained traces of nemorensine (hR_F 42) and two additional alkaloids of hR_F 24 and 15 which, however, could not be obtained in pure state.

S. nemorensis L., ssp. *fuchsii*, var. *nova* (ZLATNÍK)

The dry and ground plant (1 650 g), collected in July 1965 along the Rosenauer path at Adamov-Blansko, gave a crude mixture of alkaloids (3·5 g) and a crude mixture of alkaloids from N-oxides (1·4 g). Since these two portions were identical (thin-layer chromatography), they were combined. After purification, a mixture of alkaloids (0·47 g) was obtained. Column chromatography on aluminium oxide (12 g, column 8 mm i.d.), collection of 10 ml fractions. The fractions 1—3 (benzene–ethanol, 99 : 1) afforded substances of hR_F 80 and 76 which could not be separated because the quantity of the material was too small. The fractions 4—5 (benzene–ethanol, 99 : 1) yielded a mixture of three alkaloids of hR_F 76, 63, and 59 (9 mg). The fractions 6—12 (benzene–ethanol, 97 : 3) gave an alkaloid of hR_F 43 with traces of substances of hR_F 81 and 61. After recrystallization from ethanol, the picrate of this mixture afforded yellow needles, m.p. 226—228°C which, on the basis of the IR spectrum, were identical with the picrate of nemorensine. The fractions 13—22 (benzene-ethanol, 90 : 10) yielded an alkaloid of hR_F 24 with traces of a substance of hR_F 59. From the mixture, a crystalline picrate could not be prepared.

Isolation of the Acid *I* from *S. nemorensis* L.

The acidic ethereal extract from *S. nemorensis* L. (7·5 g) was dissolved in a saturated solution of hydrogen sodium carbonate, the solution was filtered over active charcoal, the filtrate washed 5 times with ether, acidified with sulphuric acid to Congo-red, and extracted 5 times with ether. The extract was dried and the ether was removed by distillation to give a residue (2·4 g) which on thin-layer chromatography (silica gel G, solvent system ether (10 ml)–light petroleum (10 ml)–acetic acid (5 drops)), after detection with a solution of bromophenol blue, produced three spots of hR_F 81, 60, and 45. The spot of hR_F 81 was identified as fumaric acid. Crystallization of the mixture from ethyl acetate gave 350 mg of the substance *I*, hR_F 45, m.p. 168—171°C, UV: λ_{max} 220 nm (log ε 4·12), CD spectrum: 216 and 253 nm ($\Delta\varepsilon = -1{\cdot}98$ and $-0{\cdot}18$). For $C_{10}H_{16}O_5$ (216·2) calculated: 55·54% C, 7·45% H; found: 55·38% C, 7·68% H.

Hydrolysis of Nemorensine (*V*)

A. A mixture of nemorensine (150 mg) and barium hydroxide (hydrate, 300 mg) in 5 ml of water was refluxed for 2 h. After cooling, the mixture was diluted with water, saturated with carbon dioxide, and filtered. The filtrate was acidified with sulphuric acid and extracted 5 times with 10 ml of ether. After drying and evaporation of ether, nemorensic acid (*III*, 77 mg) was obtained which crystallized from a mixture of ethyl acetate–light petroleum. M.p. 174—178°C, $[\alpha]_D^{24}$ +87° ± 3° (*c* 0·84 in ethanol), CD spectrum: 213 nm ($\Delta\varepsilon = +1{\cdot}30$). The substance does not absorb in the UV region. For $C_{10}H_{16}O_5$ (216·2) calculated: 55·54% C, 7·45% H; found: 55·29% C,

7·56% H. Mass spectrum: observed (calculated, composition): 171·1017 (171·1021, $C_9H_{15}O_3$); 153·0915 (153·1915, $C_9H_{13}O_2$); 125·0963 (125·0966, $C_8H_{13}O$); 111·0812 (111·0810, $C_7H_{11}O$); 109·1018 (109·1017, C_8H_{13}); 109·0655 (109·0653, C_7H_9O); 107·0960 (107·0861, C_8H_{11}); 83·0858 (83·0861, C_6H_{11}); 83·0492 (83·0497, C_5H_7O); 72·0672 (72·0575, C_4H_8O); 69·0703 (69·0704, C_5H_9); 69·0340 (69·0340, C_4H_5O); 67·0548 (67·0548, C_5H_7). After extraction with ether, the aqueous layer was neutralized with sodium hydroxide, the solvent evaporated under reduced pressure to dryness, and the residue extracted 5 times with ethanol. The extract was concentrated under reduced pressure to dryness and extracted again with ethanol. This procedure was repeated three times. Then the residue was extracted with hot chloroform. After evaporation of chloroform, a brown-yellow viscous substance (39 mg) was obtained which could not be brought to crystallization; crystalline picrate could also not be prepared.

B. A solution of nemorensine (50 mg) and potassium hydroxide (50 mg) in methanol (3 ml) was refluxed for 3 h, evaporated under reduced pressure to dryness, and the residue extracted 5 times with hot chloroform. After evaporation of the extract, a yellowish viscous substance was obtained whose IR spectrum differed from that of the substance prepared sub *A*, $[\alpha]_D^{24} -48° \pm 5°$ (*c* 1·0 in chloroform). The residue after extraction with chloroform was diluted with water, acidified with sulphuric acid, and extracted 5 times with ether. After drying and evaporation of ether, 17 mg of a residue was obtained which crystallized from a mixture of ethyl acetate–light petroleum to give crystals of m.p. 174—177°C which, on the basis of the IR spectrum, were identical with nemorensic acid (*III*) prepared sub *A*.

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REFERENCES

1. Müller A.: Heil- und Gewürz-Pflanzen *7*, 73 (1924).
2. Culvenor C. C. J., Koretskaya N. I., Smith L. W., Utkin L. M.: Australian J. Chem. *21*, 1671 (1968).
3. Aasen A. J., Culvenor C. C. J., Smith L. W.: J. Org. Chem. *34*, 4137 (1969).
4. Šimánek V., Klásek A., Šantavý F.: This Journal *34*, 1832 (1969).
5. Culvenor C. C. J., Woods W. G.: Australian J. Chem. *18*, 1625 (1965).
6. Bothner-By A. A.: Advan. Mag. Res. *1*, 195 (1965).
7. Cookson R. C., Crabb T. A., Frankel J. J., Hudec J.: Tetrahedron, Suppl. *7*, 355 (1966).
8. Reichstein T., Shoppee C. W.: Discussion Faraday Soc. *7*, 305 (1949).

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