1,4-SUBSTITUTED ß-CARBOLINES FROM CAREX BREVICOLLIS DC

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The paper describes new natural heterocyclic compounds, alkaloids of Carex brevicollis DC., which are 1,4-substituted ß-carbolines. There are discussed the results of studies of thier physical properties and chemical transformations which are used to determine the structure of the alkaloids and to put forward suggestions about their biogenesis.

1. Introduction

The indole compounds of the ß-carboline series are commonly found in plants, primarily, in the form of tetrahydro derivatives. Considerably fewer compounds with totally aromatic structure are known, approximately 2% of all indole alkaloids reported so far /1, 2/. Among the simple derivatives of ß-carboline (1a) the most popular ones are harman (1b), harmol (1c) and harmine(1d) / 3 /. Many new alkaloids of this

kind have been found recently. Pavettine (Ie) has been isolated from Pavetta lanceolata /4/. Alkaloids have been found which contain heterocyclic fragments as substituents - the furan one in perlomerine (If), the pyridine one in bases from genus Pauridiantha, for instance,(Ig), etc.

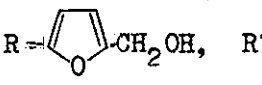
I. a. R = R′= H.

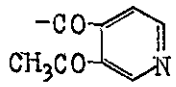
b. R = CH_3, R′= H.

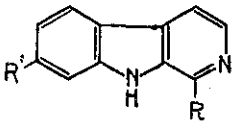
c. R = CH_3, R ′= OH.

d. R = CH_3, R′= OCH_3.

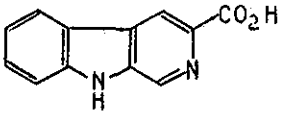
e. R = C_2H_5, R′= H.

f.  , R′= H.

g. R =  , R′= H.

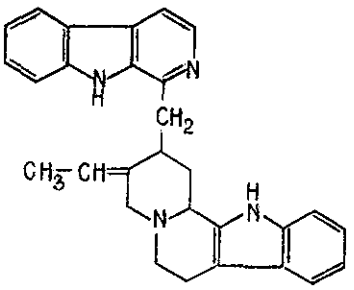


I



II

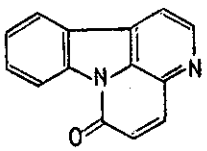
Also found in plants are the esters of the ß-carboline-3-carboxylic acid (II) tnd the complex dimer alkaloids of the type of uzambarenzine (III).



III

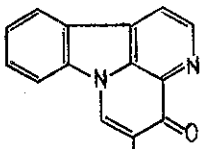
Moreover, the alkaloids with the aromatic bond system in the ß-carboline part have been found among the group of bases of the cantine type (IVa) and tuboflavine (IVb) which have a

similar structure.

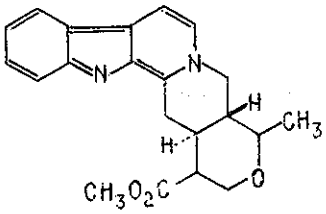


a

IV



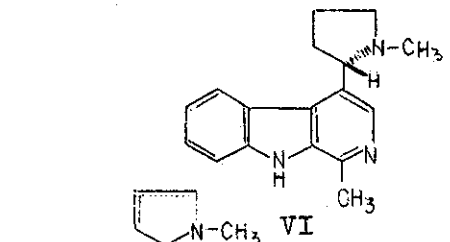
b



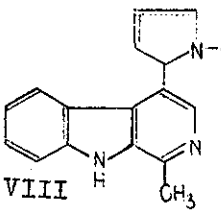
V

As we do not intend to give here complete information on the alkaloids we are interested in, we shall mention in addition only the anhydrobases found in the plants, for instance, alstonine (V).

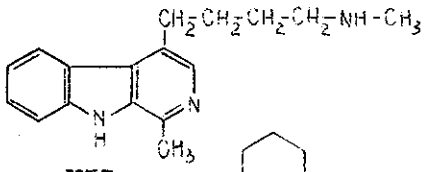
Of especial interest among the above heterocyclic compounds are the 1,4-derivatives of ß-carboline which we isolated from Carex brevicollis DC. /5/, namely, brevicolline(VI), brevicarine(VII), dehydrobrevicolline(VIII) and homobrevicolline(IX).



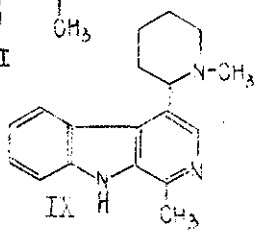
VI



VIII



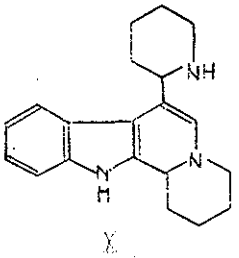
VII



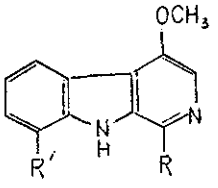
IX

The formulas show that these compounds have related structures and the first three compounds differ only in the

degree of hydrogenation of the substituent group. Brevicolline and its immediate derivatives are interesting in that they combine ß-carboline with five- and six-membered nitrogen-containing cycles. No such alkaloids have been described so far, with the exception of nitrarine with the suggested structure (X). /6/.



X



XI

a. $R=CH_2-CH_3$, $R'=H$.
b. $R=CH_2-CH_3$, $R'=OCH_3$.
c. $R=CH=CH_2$, $R'=H$.

Substitution at the fourth position of ß-carboline is not typical of the natural compounds. Among these few compounds there are also crenatine(XIa) and crenatidine (XIb) isolated from Aeschrion crenata and the vinyl analogue of crenatine (XIc) isolated from Picrasma javanica /7/.

The novelty of the alkaloids of Carex brevicollis and the fact that they are found only in one plant species suggest that a review of their chemical properties, transformations and syntheses will be of interest for the chemists who work with the heterocyclic compounds.

II. General information on Carex brevicollis DC.

This grassy plant which belongs to the Cyperaceae family is common in the south-western part of the USSR. It grows in beech, hornbeam and ash woods; its mode of reproduction is, primarily, vegetative though reproduction with seeds is of some importance, too. The plant is hardy and can be readily cultivated. A plantation, once established, can be exploited for several years without replanting since the cut leaves soon

grow again. The mean yield of alkaloids in the favourable period is 0.65% calculated for absolutely dry plant mass.

The total of seven alkaloids has been found in the plant, brevicolline(VI) and brevicarine(VII) which comprise 90% of the total content of the alkaloids are the most important among them.

The plant is first treated with ammonia and then the alkaloids are extracted with chloroform or dichloroethane. The extracts are treated with diluted sulphuric acid to transform alkaloids into water-soluble sulphates, and then the free bases are sedimented with ammonia. Brevicarine is isolated in the form of basic salt which is poorly soluble in water. The mixture of alkaloids is further treated with boiling methanol. Cooling of the solution leads to crystallization of brevicolline, the minor alkaloids remain in the mother liquour and brevicarine is in the insoluble residue.

To isolate brevicarine, the residue which is insoluble in methanol is treated with 5% sulphuric acid, clarified with carbon and filtered. The filtrate is alkalized with sodium hydroxide and the isolated alkaloid is crystallized from acetone or chloroform.

The minor alkaloids are divided according to the degree of basicity using further various chromatographic techniques.

Another method is to extract alkaloids from the plants with 2% sulphuric acid. The acidic extracts are treated with ammonia and completely extracted with chloroform. The following procedures are carried out as described above.

The proportions of various alkaloids in the mixture depend on the plant's age, its development stage and its growth

location. The mean proportions of the alkaloids are given in Table I along with the most important physical properties of the alkaloids.

Table I

Alkaloid	Formula	Melting point		$[\alpha]_D$	Mean content in mixture (%)
		Base	Dihydrochloride		
Brevicolline	$C_{17}H_{19}N_3$	224 - 5	273 - 4	-145.8	55.5
Brevicarine	$C_{17}H_{21}N_3$	110 - 2	195 - 6	-	36.5
Dehydrobrevicolline	$C_{17}H_{17}N_3$	237 - 8	278 -80	-25	3.0
Homobrevicolline	$C_{18}H_{21}N_3$	261 - 3			5.0
Harman	$C_{12}H_{10}N_2$	238			
Harmol	$C_{12}H_{10}ON_2$	321			
Harmine	$C_{13}H_{12}ON_2$	256			

Three Carex alkaloids which contain 17 carbon atoms in the molecule and homobrevicolline are solids which precipitate as well-formed crystals. They are insoluble in water but soluble in aqueous acid solutions, odourless and have bitter taste. These alkaloids are biacidic bases; the ionization constants of brevicolline and brevicarine were determined by means of potentiometric titration in dimethylformamide /8/; for brevicolline it was determined pK_{a1} 7.12 and pK_{a2} 4.48 and for brevicarine, respectively, 9.43 and 4.05.

The alkaloids give rise to two series of salts both with mineral and organic acids. The diluted solutions of the salts of VI, VIII and IX possess intensive blue fluorescence and the salt of VII possesses violet fluorescence.

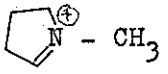
III. The structure of brevicolline, brevicarine and dehydrobrevicolline

The initial information on the structure of the Carex alkaloids has been obtained by comparing their UV spectra with the spectrum of harman. Both the neutral and acidic solutions of these alkaloids have been found to possess identical absorption patterns. The following values of λ_{max} (nm)(lg ε) have been found for the compound VI: 242 (4.42), 288 (3.98), 338 (3.64), and 352 (3.69). In the acidified solutions each of the maxima is shifted by a certain interval towards the long-wavelength region. These data suggest that the compounds have common chromophore, namely, the ß-carboline fragment.

The analysis of the IR spectra has yielded data on the nature of the atom groups. The spectra have many similar features, primarily, the band typical of the ortho-substituted derivatives of benzene. This band is found at 740 cm^{-1} in the spectra of the compounds VI and VIII and at 770 cm^{-1} in the spectrum of the compound VII. Absorption in the 3440 - 3400 cm^{-1} range indicates the NH group of the indole ring. The bands at 1625, 1575 and 1460 cm^{-1} correspond to the vibrations of the benzene and pyridine rings and the bands at 1160, 1060, 910 and 670 cm^{-1} correspond to the CH groups of the same rings. The absorption difference between the alkaloids is most clearly seen in the range between 1110 and 1000 cm^{-1} and at 1200 cm^{-1}. These absorption bands correspond to the fourth ring in the compounds VI and VIII while the compound VII has practically no absorption at this range. The additional difference is the band at

810 cm^{-1} for the compound VIII.

The formulas of alkaloids are substantiated by the molecular ion signals at m/e 265 (VI), 267 (VII) and 263 (VIII). The maximum intensity peak in the mass spectrum of the compound VI is at m/e 84. The same signal is found in the spectrum of nicotine, it is attributed to the fragment

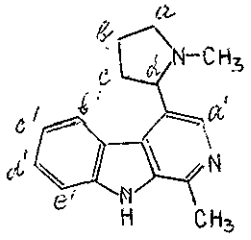
 which is produced due to the α-rupture of the C - C bond between pyrrolidine and pyridine rings /9/. A similar rupture seems to occur in the case of the compound VI. Fragmentation of the compound VIII is similar to that of the compound VI. The maximum peak in this case is found at m/e 82 which agrees with the presence of the pyrroline ring. In the high-mass region there are registered the signals of the fragments M - 1 and M - 29 which are due to removal of the hydrogen adjacent to N - CH_3 and the ethyl radical formed following opening of the pyrrolidine ring. It is more difficult to account for the fragment M - 43 since it necessitates rupture of several bonds. Nevertheless, it is found in the mass spectra of the compounds with the pyrrolidine bond system. /9/. In the low mass number region it corresponds to the signal m/e 42 which must be due to the stable structure $CH_3 - \overset{+}{N} \equiv CH$.

The mass spectrum of brevicarine(VII) shows after the molecular peak the ions with the masses M - 15, M - 30, M - 44, M - 58 and M - 72. Their appearance is due to sequential rupture of the C - C bonds of the open alkylamine chain as is the case with electron impact in the molecules of aliphatic amines. In the low-mass region the maximum-

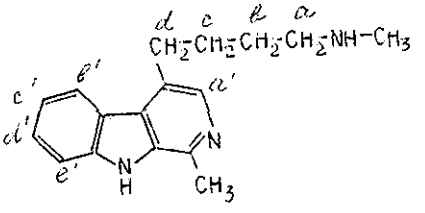
intensity signal at m/e 44 seems to correspond to the ion $CH_3 - \overset{+}{N}H = CH_2$.

For all three alkaloids the aromatic part of the molecule gives weak signals in the spectra owing to the stability of this fragment.

The PMR spectra of brevicolline and brevicarine are clearly similar owing to the structural similarity between these alkaloids but also there are some differences which reflect the peculiarities of structure. The integral curves show that the spectra represent all the hydrogen atoms of the alkaloids studied. The low-field spectra for both alkaloids exhibit groups of lines of the aromatic protons.



VI



VII

According to the chemical shifts (δ 7.50 ppm) one of the lines is attributed to the protons c′, d′ and e′. They are equivalent and their signals are in the same region. The signals of the protons b′ are found at 8.35 ppm for the compound VI and 7.95 ppm for the compound VII. The larger shift in the compound VI is due to the effect of not only the benzene ring but of the proximate pyrrolidine ring while the alkylamine chain in the compound VII exert no such strong effect.

The presence of the signals of the α hydrogen atoms belonging to the pyridine ring (the protons a′ with the chemical

shifts 8.55 and 8.10 ppm in the spectra of the compounds VI and VII) strongly suggests 1,4-substitution in the ß-carboline fragment.

According to the chemical shift values the high-field peaks have been attributed to the aliphatic protons. For instance, the singlet at 2.80 ppm which is found in both spectra is attributed to the protons of the CH_3 group bonded to the carbon of the pyridine ring and the peaks at 2.25 and 2.35 ppm have been attributed to $N - CH_3$ in the compound VI and $NHCH_3$ in the compound VII. Satisfactory identification has been done also for the methylene protons a, b and c in the above formulas. The proton d corresponds to the signal at 3.90 ppm in the spectrum of the compound VI. Brevicarine has at this site the group CH_2 bonded to the aromatic ring, the signal of its protons is at 3.10 ppm.

The signal at 10.70 ppm has been clearly identified as that of the proton of the NH indole group since this signal was shifted to 9.74 ppm when the spectrum was recorded at the temperature increased by 30 °C and this corresponds to the chemical shift data. Thus, the spectral characteristics of the Carex alkaloids make possible well-founded conclusions about their structure. A study of their chemical properties discussed in the following section supports the above conclusions.

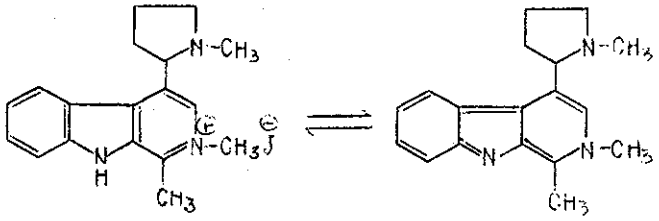
IV. The chemical properties of the Carex alkaloids

1. Alkylation at nitrogen

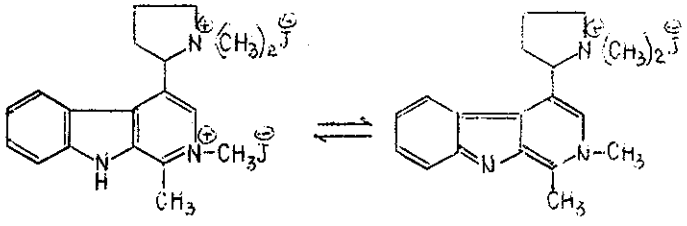
The first stage of alkylation of the carboline derivatives gives rise to the products which convert under the effect of alkalies into anhydrobases of bright yellow colour /10/.

The methyl iodides of the Carex alkaloids treated with sodium hydroxide also give rise to coloured compounds of a similar structure.

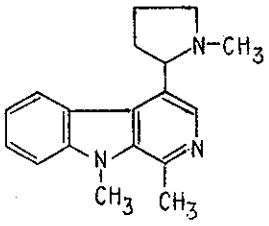
Varying the conditions of methylation various reaction sequences can be carried out. For instance, at the room temperature in the acetone solution brevicolline reacts with methyl iodide at the nitrogen of the pyridine ring giving rise to the compound XII which corresponds to the anyhdrobase XIII. When the compound VI and CH_3I are heated in methanol alkylation of the amino group of the pyrrolidine group (XIV) occurs. The alkaloids which have hydrogen at the indole nitrogen react with potassium hydroxide in boiling xylene giving rise to potassium derivatives. These intermediate compounds lead to ind-N-methyl compounds (XV). The complete methylation product is the compound XVI.



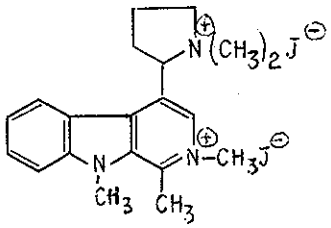
XII XIII



XIV

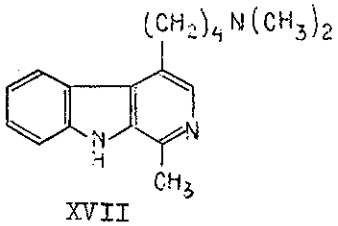


XV

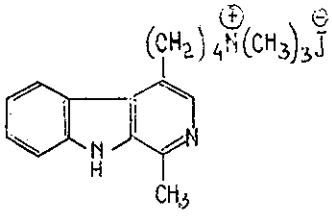


XVI

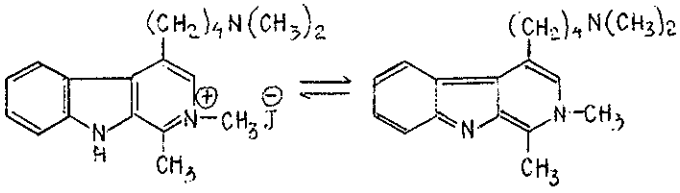
Since brevicarine contains the group $NHCH_3$ it gives rise to methylbrevicarine (XVII) according to Wallach. The complete methylation reaction proceeds as described above giving rise to all the possible products (XVII - XXII).



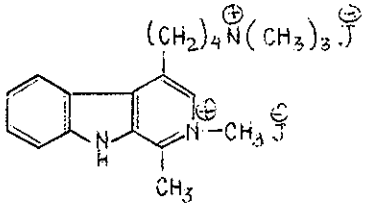
XVII



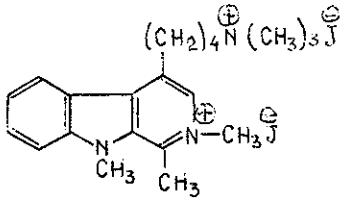
XVII



XIX XX



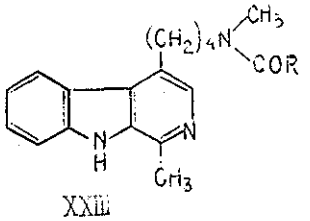
XXI



XXII

2. Acylation

The acylation reaction is typical of the Carex alkaloids. Acylation of brevicarine occurs via the usual pathway. For instance, the reaction between brevicarine and acetic anhydride readily gives rise to the N-acetyl derivative and benzoylation of brevicarine occurs under the conditions of the Schotten-Baumann reaction (XXIII a and b).

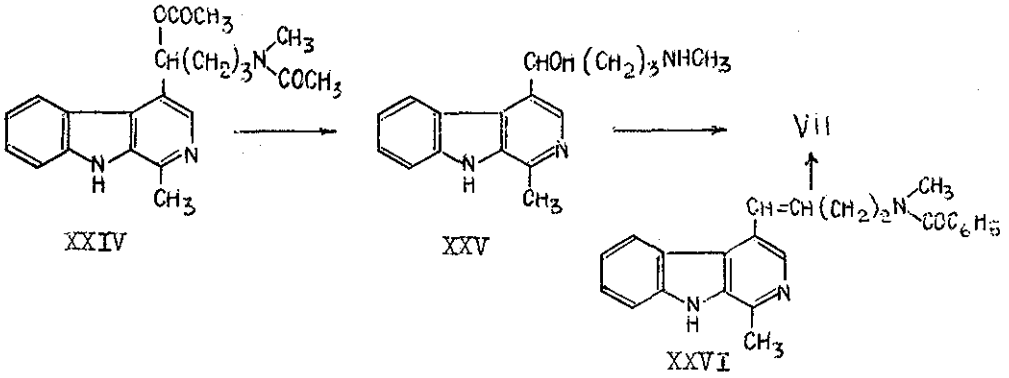


XXIII

a. R = CH_3

b. R = C_6H_5

When brevicolline is treated with the same reactants the reactions involve opening of the pyrrolidine ring. Boiling of the compound VI with acetic anhydride at the first stage yields O,N-diacetate (XXIV) which is converted by means of alkaline hydrolysis into amino alcohol (XXV). Heating of the alkaloid with benzoyl chloride gives rise to unsaturated N-benzoyl derivative XXVI.



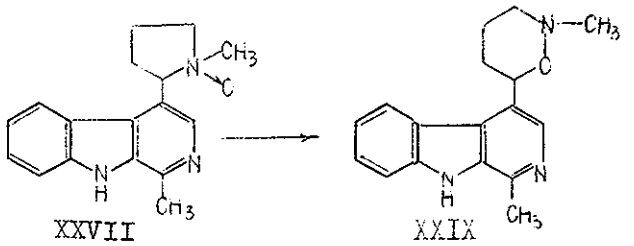
XXIV XXV XXVI

The acylation reaction made it possible to convert brevicolline into brevicarine, for instance, by debenzoylation and hydrogenation of the compound XXVI. The rezulting compound VII was identical with the compound isolated from the plant in all its properties.

3. Formation and decomposition of the N-oxides

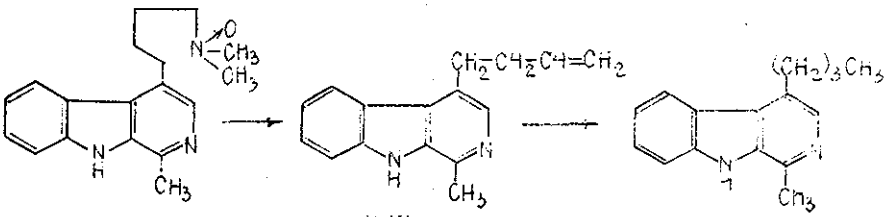
The reactions between brevicolline(VI) or N-methylbrevicarine(XVI)with the concentrated hydrogen peroxide in methanol in the presence of sodium tungstate give rise to the respective N-oxides (XXVII and XXVIII). These solids crystallize from aqueous acetone as thin needles. The pyrogenic decomposition reaction gives rise to deamination of alkaloids.

At the first stage of the reaction the N-oxide of brevicolline undergoes regrouping /11/ giving rise to the oxazine derivative (XXIX) whose reduction over the Adams catalyst produces amino alcohol (XXV) which is identical with that produced by hydrolysis of diacetate (XXIV). Hydrogenolysis of this amino alcohol gives rise to brevicarin. This is another of the reactions which illustrate the relationship between the Carex alkaloids.



XXVII XXIX

Deamination occurs at the decomposition stage of the compound XXVIII with heating in vacuum. This gives rise to 4-butenylharman (XXX) whose melting point is 181 - 182 °C (from acetone). The catalytic hydrogenation of this compound over platinum dioxide results in addition of 1 mole of hydrogen giving rise to 4-butylharman whose melting point is 207 - 208 °C (from acetone)(XXXI).



XXVIII XXX XXXI

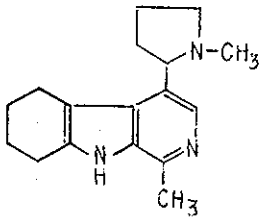
We managed to compare this product with the compound produced by dehydrogenation of brevicolline over selenium and to demonstrate their identity.

4. Hydrogenation and dehydrogenation

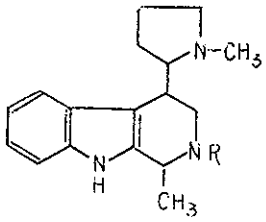
Hydrogenation of brevicolline proceeds via different pathwaus according to the conditions. As was expected according to the results of Schwarz and Schlittler /12/, in the acetic acid solution over platinum dioxide the reaction occurs at the benzene ring leading to crystallization of 5,6,7,8-tetrahydrobrevicolline(XXXII) from acetone, the melting point 234 - 237 °C, $[\alpha]_D$ −85 °. The UV absorption spectrum exhibits the following values of λ_{max} (nm)(lg ε): 227 (5.69), 278 (4.86) and 302 (4.91) in alcohol. The band at 740 cm^{-1} in the IR spectrum is absent. The molecular ion in the mass spectrum: M^+ 269.

Treatment of methiodide of brevicolline(XII) and the respective chlorobenzylate with sodium borohydrid results in hydration of the pyridine fragment giving rise to 1,2,3,4-tetrahydro derivatives(XXXIII a and b). This result agrees with the earlier reported data on the reactions with simpler carbolines /13/. 1,2,3,4-Tetrahydrobenzylbrevicolline (XXXIII) is a crystalline solid with the melting point of 166 - 167 °C (from methanol). Its UV spectrum is typical of the compounds with the indole chromophore. The IR absorption

spectrum lacks the band at 1600 cm^{-1} which is typical of the pyridine ring bonds. The molecular ion in the mass spectrum: M^+ 359.



XXXII



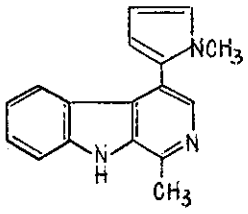
XXXIII

a. R = CH_3

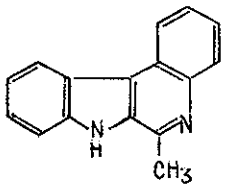
b. R = $CH_2 - C_6H_5$

The Carex alkaloids are dehydrogenated when heated over the palladium charcoal. The compound VI gives rise to pyrroloharman (XXXIV) due to the reaction which occurs at the pyrrolidine ring. Nicotine undergoes a similar transformation under the action of some dehydrating agents. In contrast to the starting brevicollin, 4-(N-methyl- ⍦ -pyrryl)harman is a monoacid base since the pyrrole nitrogen lacks the basic properties; potentiometric titration in anhydrous acetone exhibits only one potential drop.

A side product of dehydrogenation of brevicolline is 3,4-benzoharman (XXXV). The initial stage of the process seems to be the opening of the pyrrolidine ring, then deamination is accompanied by ring formation and, finally, aromatization occurs.



XXXIV



XXXV

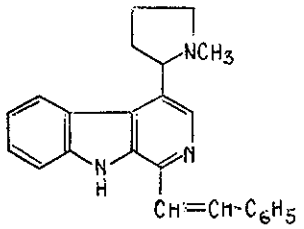
Similar cases have been reported in literature /28/. This reaction mechanism agrees with the results of dehydration of the compound VII which gives rise to only the compound XXXV under similar conditions.

It is appropriate to remember that the minor alkaloids of Carex include dehydrobrevicolline(VIII). However, we failed to find it or a similar compound in the products of dehydrogenation of brevicolline.

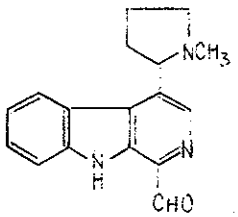
5. Oxidation

The Carex alkaloids are readily oxidized giving rise to complex mixtures which are hard to separate into individual compounds. However, some of the reactions yield valuable results. Heating of brevicollinewith concentrated nitric acid gives rise to 3,5-dinitroanthranilic acid. Under the conditions of the Kuhn - Roth reaction there occurs removal of 1 mole of acetic acid. This reaction confirms the presence of the group C - CH_3 in the molecule.

The fact that this group is adjacent to the nitrogen of the pyridine ring has been confirmed in the reaction with benzaldehyde; when it is boiled with brevicollinethe benzylidene derivative (XXXVI) is produced. Oxidation with selenium dioxide gives rise to the aldehyde XXXVII.

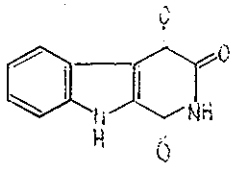


XXXVI

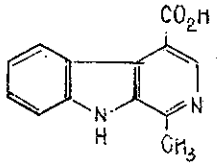


XXXVII

Oxidation of brevicolline with chromium trioxide in aqueous solution of sulphuric acid gives rise initially to a golden yellow chromate sediment which is gradually dissolved with heating; the processes of oxidative decomposition occur and the solution turns green. The following products of the reaction have been identified: N-succinimide, 1,3,4-triketo-1,2,3,4-tetrahydro-ß-carboline (XXXVIII) and harman-4-carboxylic acid (XXXIX).



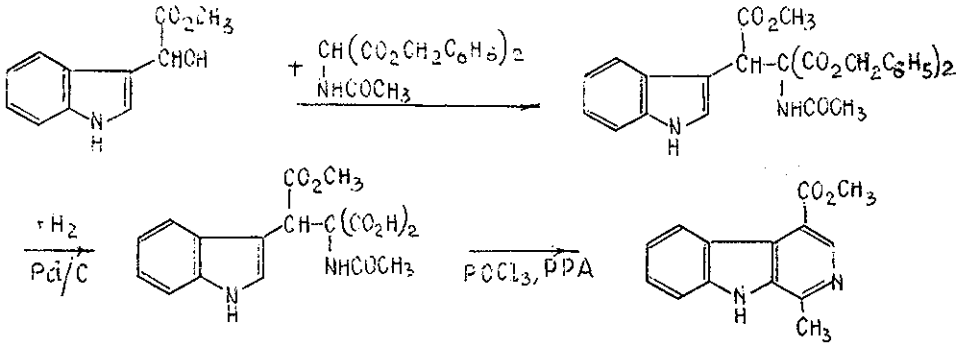
XXXVIII



XXXIX

The triketo-derivative has been identified by its IR spectrum which exhibits three intensive bands (at 1740, 1660 and 1620 cm^{-1}) which are typical of the -CO- and -CO-NH-CO- groups.

The heating of the acid XXXIX in vacuum up to the melting point results in its decarboxylation giving rise to harman (Ib). To confirm the structure of the acid its methyl ester has been synthesized according to the following scheme:

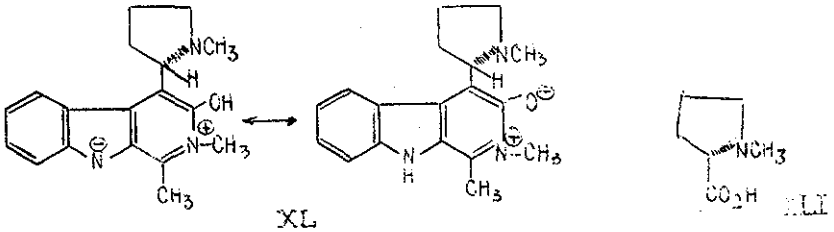


The methyl ester of the indolylglycolic acid has been produced by reduction of the indolylglyoxylic ester with amalgamated aluminium. Condensation and hydrolysis have been carried out according to /14/. The remaining processes have been carried out in one stage using phosphorous oxychloride in polyphosphoric acid.

The methyl ester of the harman-4-carboxylic acid synthesized in this way and the ester produced by oxidation of brevicolline have proved to be identical.

This oxidation reaction was repeated by Kompish et al. /15/ in their study of brevicolline biosynthesis.

Blaha et al. /16/ have carried out oxidation of brevicolline methyl iodide (XII) with potassium ferricyanide to determine the absolute configuration:



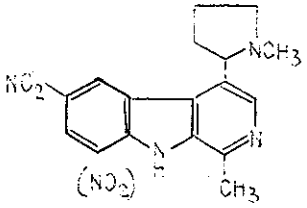
XL XLI

The reaction gives rise to the compound XL for which the UV, IR and PMR spectra have been recorded and its picrate has been analyzed. This product has been further oxidized with chromium trioxide to the optically active hygric acid (XLI). The S configuration of the asymmetric centre of brevicolline has been confirmed by direct comparison with the L-hygric acid produced from L-proline and by comparing with the spectropolarometric results for (-)-nicotine, (-)-methylanabasine and (-)-brevicolline.

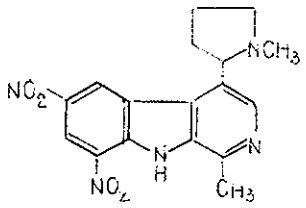
6. Nitration

Nitration reactions for brevicolline, brevicarine and their N-methyl-, N-acetyl- and hydroxy derivatives have been studied /17/. Nitration was carried out using the nitric acid (specific weight 1.52) in the presence of concentrated sulphuric acid. The reaction with brevicolline gave rise to 6- and 8-mononitro- and 6,8-dinitro compounds while brevicarine yielded only the 6,8-dinitro derivative (XLII, XLIII and XLIV).

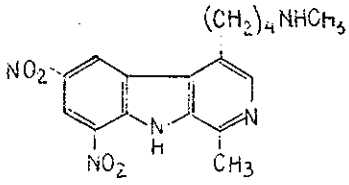
Interestingly, nitration of the hydroxy derivative of brevicarine is accompanied by concurrent ring formation giving rise finally to the compound XLII. This illustrates transition from compound VII to VI.



XLII



XLIII



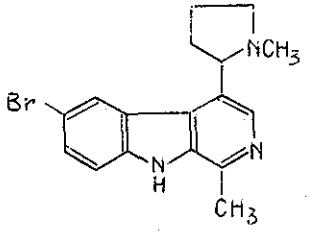
XLIV

All the nitro compounds have intensive colouration varying from bright yellow to red. This seems to be due to possible formation of quinoid structures. This is confirmed by the fact that the mass spectra of 6-nitro- and 6,8-dinitro derivatives exhibit the fragment M - 17 whose formation must be attributed to removal of the OH group.

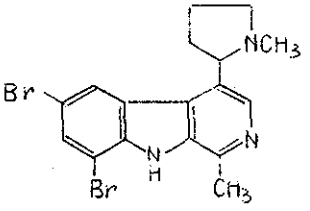
7. Bromination

Bromination of the hydrochloride of the compound VI with bromine in acetic acid /18/ gives rise to 6-bromo- and 6,8-dibromobrevicolline (XLV and XLVI). Bromination of

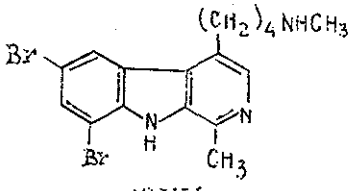
brevicarine yields three compounds, namely, di- and tribromobrevicarine (XLVII and XLVIII) and 6-bromobrevicolline (XLV). The latter may be produced from a hypothetical intermediate bromoamine which we could not find in the reaction products.



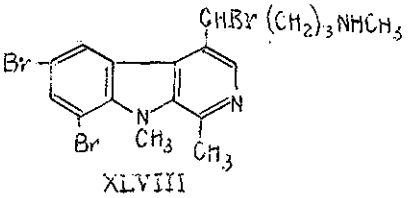
XLV



XLVI



XLVII



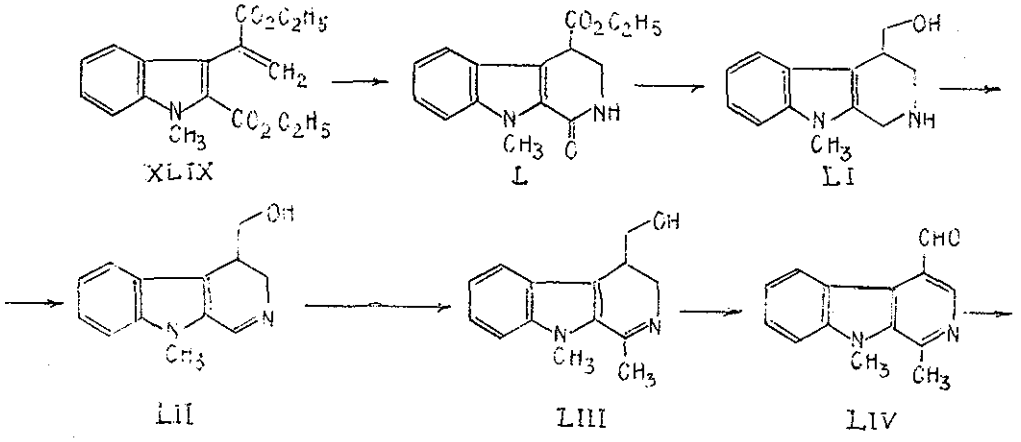
XLVIII

The structures of the bromides of the Carex alkaloids have been confirmed by the UV, IR and mass spectra.

8. Total synthesis of methylbrevicolline

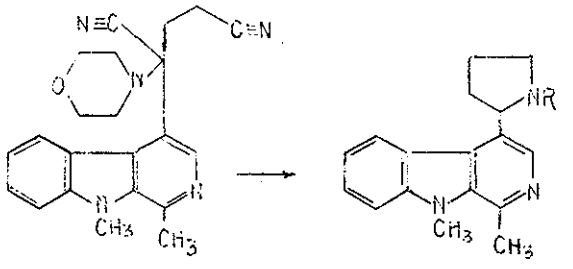
Methylbrevicolline was synthesized to illustrate production of 1,4-substituted ß-carbolines /19/. The starting compound was the ester XLIX which had been produced by condensation

of methylphenylhydrazine with the ethyl ester of the pyroracemic acid. The compound XLIX converted with ammonia into the respective lactam (L). Reduction of the compound L with lithium aluminium hydride gave rise to the compound LI. Oxidation of this amino alcohol with lead tetracetate gives rise to dihydro-ß-carboline (LII) which is readily alkylated at the position 1 with the Grignard reactant. Oxidation of the compound LIII yields the aldehyde LIV.



XLIX L LI

LII LIII LIV



LV LVI

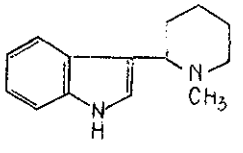
a = H
ß = CH_3

This aldehyde is a key compound for synthesizing the 4-pyrrolidine derivatives of ß-carboline, including 2-methylbrevicolline. The reaction with morpholine and cyanide ions (the Michael addition) yielded morpholinodinitrile (LV). Acidic hydrolysis of this product produces ketonitrile via reducing ring formation which gives rise to the compound LVI a which yields N-methylbrevicolline(racemate) LVI b following methylation with formaldehyde and cyanoborohydrid. The base LVI b is identical with methylated brevicolline isolated from plants as evidenced by spectroscopic results and thin-layer chromatography.

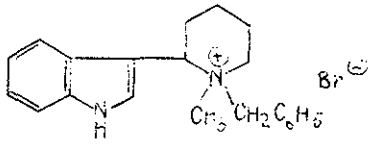
9. Synthesis of brevicarine

An earlier study /20/ shows that the Beckman regrouping of oximes of the ß-(indolyl-3)-ketones produces 4-substituted 3,4-dihydro-ß-carbolines which can be dehydrated yielding the respective ß-carbolines.

This reaction was used also for synthesizing brevicarine and its lowest homologue /21/. The starting compound in synthesis of brevicarine was piperidylindole LVII which was produced by condensation of indole with 1-methylpiperidone-2 according to Powers /22/.

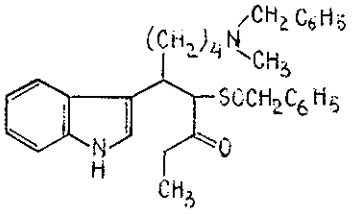


LV11

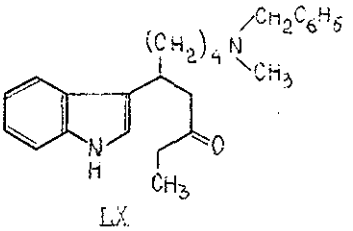


LV111

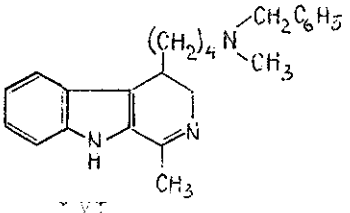
The compound LVII is treated with benzyl bromide yielding the quaternary salt LVIII. Condensation of this gramine analogue with potassium derivative of benzylsulphanilacetone gives rise to ß-ketosulphoxide LIX which yields the ketone LX following treatment with amalgamated aluminium in aqueous tetrahydrofuran. The oxime of this ketone underwent the Beckman regrouping under the action of phosphorus pentachloride in nitrobenzene. The process was accompanied by concurrent ring formation and gave rise to the dehydro derivative LXI. Finally, dehydration of this product using the palladium black in ethylene glycol produced brevicarine.



LIX



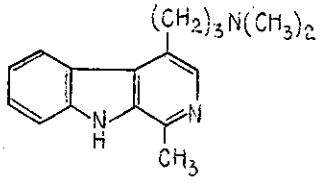
LX



LXI

As evidenced by the IR and mass spectra and by the temperatures of melting of the mixed samples, the resulting base and its dihydrochloride are fully identical with the natural brevicarine and its hydrochloride.

A similar process produced also the N-methyl derivative of the lowest homologue of brevicarine(LXII).



LXII

V. Qualitative and quantitative determinations

The alkaloids of Carex brevicollis DC. are determined using the standard techniques and also their fluorescence under UV irradiation. If a drop of sap from a fresh plant leaf is put on paper and observed through a filter which absorbs the visible light one can see a luminous spot. This provides a simple and easy technique for distinguishing this plant from others with similar morphological structure.

Qualitative determinations of brevicolline and brevicarine in the extracts are made using the standard paper chromatographic techniques for alkaloid determinations or in a thin layer of aluminium oxide of the third degree of activity with the chloroform - methanol or benzene - methanol (95:5) systems. The moist chromatogram is sprayed with the Dragendorf reagent (this gives rise to orange-coloured spots) or observed under UV light when the alkaloid spots have intensive blue fluorescence.

Quantitative determinations are made with a modified spectrophotometric technique /23/ based on the UV absorption properties of the salts of the alkaloids. In contrast to the free bases which have identical absorption spectra, the salts differ by their absorption and, what is very important, their absorption maxima have different relative shifts. Hence, the absorption curves for brevicolline and brevicarine intersect at various points (see Fig. 1). The intersection point at λ = 256 nm has been chosen as the reference point for

analysis. Since both bases have identical concentrations at this point which can be found from the formula $C = \frac{D}{\varepsilon}$, we can calculate the value of ε from the optical density values for the solutions of known concentrations and thus determine the total content of these alkaloids in solutions of various mixtures or extracts from plants.

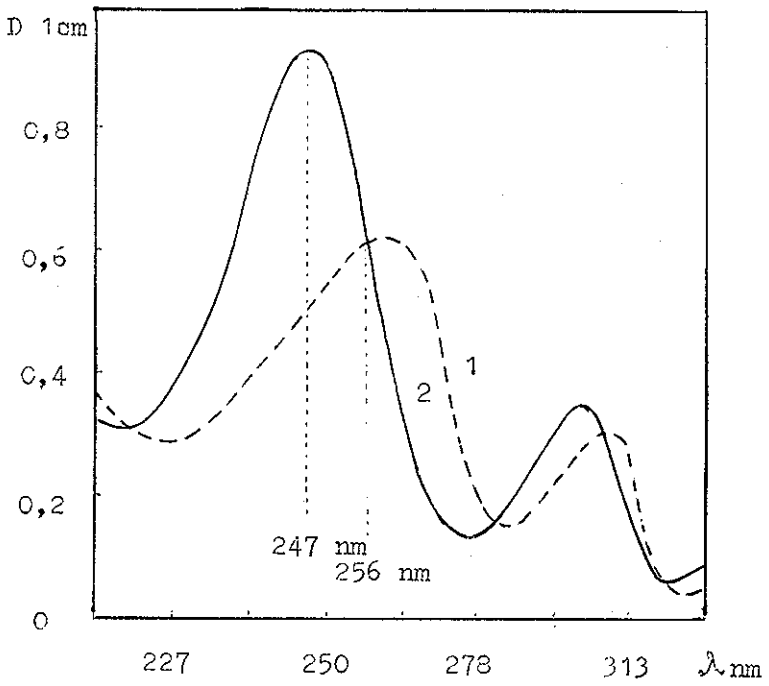


Fig. 1. The absorption curves for brevicolline and brevicarine (1 and 2) in hydrochloride solution.

The ratio between the optical densities of solutions for given wavelengths is known to be a constant. This makes it possible to determine the proportions of the components in solutions of mixtures. Fig. 1 indicates that the difference between the optical densities of our compounds is the highest at 247 nm; hence, this point is the best for analytical

purposes. The experimental determinations yielded $dD = \frac{D_{247}}{D_{256}} =$ = 1.60 for pure brevicarine and 0.80 for brevicollin. Then the formula $\frac{(1.60 - dD_{mix.}).100}{0.80}$ was used for calculating the content of brevicolline in the mixture; the only experimental determinations required for that were measurements of the optical density of the solution studied at two points.

The ratio between these alkaloids can be found using the calibration plot which is prepared using the optical densities of the solutions of pure compounds and their known mixtures for given wavelengths. This plot is straight line which connects two points on the axes of ordinates for brevicolline and brevicarine ($dD = \frac{D_{247}}{D_{256}}$) (see Fig. 2).

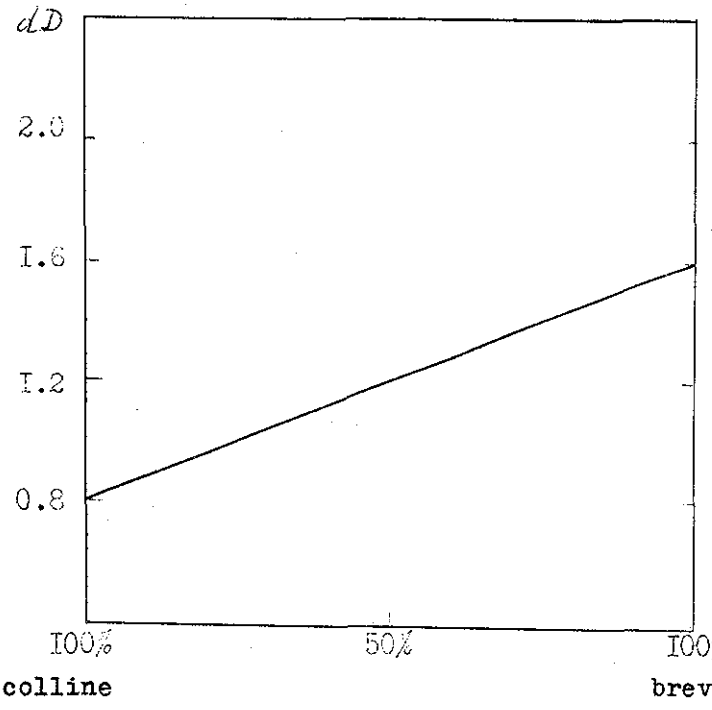


Fig. 2. Calibration plot for determination of the brevicarine: brevicolline ratios in the mixtures.

The effect of the minor alkaloids in the mixture on the determination results was not taken into consideration. Due to the low content of the minor components their effect was slight. The control determinations showed deviations in the brevicolline content of no more than 2.5%.

VI. Biochemistry

The above determination techniques have been used to study various factors and external effects which influence accumulation of the alkaloids by the plant, proportions of the main alkaloids and their distribution over individual parts of the plant.

It has been found that the total content of alkaloids practically does not depend on the location or conditions of growth of the plants. The specimens collected from different territories of natural habitat and from cultivated plantations revealed no difference in the content of accumulated alkaloids.

However, the Carex plants exhibit considerable ontogenetic variation as regards the content of alkaloids. The seeds have a small alkaloid content, as low as 0.17% for dry weight. But even in the seedlings the alkaloid content increases by a factor of tens. Young leaves also exhibit an increased alkaloid content. The ratio between the components also varies. While the seeds contain equal amounts of brevicarine and brevicolline the content of brevicolline increases in the leaves, sometimes up to 90%. In the mature leaves the mean brevicolline content varies from 55 to 60% of the total alkaloid content which is typically 0.5 - 0.6% by the dry weight. The roots have a low alkaloid content (0.20 - 0.25%) and they exhibit

a higher proportion of brevicarine - up to 70 - 75% of the total alkaloid content.

The active synthesis of alkaloids in most plants is known to occur in the roots /24/ from where the resulting alkaloids are transported to other plant parts where they are accumulated, transformed or disintegrated. If this is the case in Carex plants it can be suggested that brevicarine is the primary alkaloid. It is just brevicarine which dominates in the root cells, that is, its synthesis is predominant here. Further, the alkaloids are transported with the cell sap to the leaves where they accumulate in different proportions with the increasing share of brevicolline. This assumption was substantiated by the experiments on infiltration of brevicolline or brevicarine into the plant via the root system. Initially, the content of alkaloids in the leaves increased but later it was levelled out to the normal level, apparently, owing to the effect of an enzyme system which regulated the content and ratio of alkaloids.

Possible transformation of brevicarine into brevicolline was demonstrated by growing Penicillium on the standard culture media containing brevicarine /25/. This transformation due to closing of the alkylamine chain of the alkaloid producing pyrrolidine ring was repeatedly observed in chemical transformations of brevicarin. Finally, we could observe ring formation due to prolonged irradiation of the solution of brevicarine hydrochloride with the UV light. Interestingly, this process gave rise, finally, to a mixture containing only 2/3 of brevicolline. This ratio between the components is observed also in the plant leaves.

It is assumed that in biosynthesis the ß-carboline skeleton is produced by condensation of tryptophan with acetaldehyde or its biochemical equivalent, namely, the pyroracemic acid /26/. We can mention the glutamic acid as one of the precursors of the pyrrolidine ring and the formic acid as the contributor of the methyl group.

These compounds labelled with ^{14}C were used in the experiments on biosynthesis of brevicolline/15/.

The radioactive brevicolline with the relative content of 0.01% was isolated from the Carex brevicollis plants grown on nutrient solution with the addition of labelled tryptophan (DL-2-^{14}C). Its oxidation yielded harman-4-carboxylic acid which retained practically the total radioactivity of brevicolline. Decarboxylation of the acid gave rise to harman with 92% of the initial activity and carbon dioxide which contained practically no label. These results and the location of the label in the starting tryptophan show that the labelled carbon is at the position 3 in the ß-carboline skeleton of brevicolline.

The labelled sodium pyroracemate (2-^{14}C) also gave rise to labelled brevicolline and the label was concentrated at the C-methyl group. Oxidation according to Kuhn-Roth gave rise to acetic acid with 91% of the activity of brevicolline while decomposition of sodium acetate gave rise to barium carbonate with 98% of the activity of acetic acid and inactive methylamine hydrochloride. Thus, these results testify to inclusion of the CH_3-^{14}C fragment of the pyroracemic acid into the ß-carboline system of brevicolline with the label at the position 1.

On the whole, formation of the ß-carboline system of brevicolline agrees with accepted concepts on biosynthesis of the alkaloids of this group.

Experiments with labelled sodium formate have shown that it acts as the precursor of the N-methyl group. However, the experiments with the universally labelled glutamic acid produced ambiguous results yielding no suggestions about its participation in formation of the pyrrolidine ring.

When labelled lysine (L-2-^{14}C) was added to the nutrient solution it was determined only in which of the Carex alkaloids this acid is primarily incorporated. To do this the radioactive precursor was introduced via the root system to the plant and then the alkaloids were isolated from it. The paper chromatography was used to separate the aliquot of the solution preparatively into individual compounds and then the activity of the appropriate sites was measured. The predominant label content was found in one of the minor alkaloids (over 75% of the total amount).

This compound was found earlier among the Carex alkaloids though in small quantities. When lysine was added to the nutrient solution its content was markedly increased. This compound was isolated, its molecular weight was found to differ from that of brevicolline by 14 units (mass-spectrum) and the primary signals of the fragmentary ions are shifted accordingly. The nature of fragmentation is similar to that of brevicolline. The compound was called homobrevicolline and it has been suggested that it is the piperidine analogue of the principal alkaloid of Carex brevicollis DC. (IX). This

suggestion is supported by the well-known fact that lysine is the precursor of the piperidine fragment of many alkaloids.

VII. Pharmacology

The specific pharmacological effect of the alakloids of Carex brevicollis DC. is their stimulating effect on the contraction function of the smooth muscles /27/. This effect is especially marked in the case of brevicolline owing to which it is used in medical practice as an obstetrical drug. Monohydrochloride of brevicolline is used in the form of 1% aqueous solution for injections. There are some prospects for using the drug in veterinary practice for treating infertility of cattle.

Brevicarine dihydrochloride has a weaker effect on the smooth muscles than brevicolline and therefore it has no applications yet.

REFERENCES

1 M. Hesse, "Indolalkaloide in Tabellen", Springer Verlag, Berlin, 1968.

2 V. Snieckus, "The alkaloids", Vol. XI, ed. by R.H.F. Manske, Academic Press, New York, 1968.

3 H. Boit, "Ergebnisse der Alkaloidchemie bis 1960". Acad. Verlag, Berlin, 1961.

4 R. Raffauf, "Handbook of Alkaloids and Alkaloidcontaining Plants", Wiley Interscience, New York, 1970.

5 G. Lazurjevski, I. Terentjeva, "II Indo-Soviet symposium on the chemistry of natural products", New Delhi, 1970.

6 M. Normatov, S. Yunusov, Khim.Prir.Soedin., 1968, 139.

7 E. Sanches, J. Comin, "The Chemistry of natural products" IUPAC, Stockholm, 1966.

8 T. Shirshova, I. Terentjeva, Izv. Akad. Nauk MSSR, Ser. Biol.,Khim.,1973, 2, 76.

9 H.Budzikiewicz, C.Djerassi, D.Williams, "Interpretation of Mass spectra of organic Compounds", Ed."Mir", Moscow, 1966.

10 A. Katritzky (ed.) "Advances in heterocyclic Chemistry", Vol.3, Acad. Press, New York - London, 1964.

11 A. Cope, E. Trumbuce, "Organic reactions", Vol. 11, John Wiley and Sons, New York - London, 1960.

12 H. Schwarz, E. Schlittler, Helv., 1951, 34, 629.

13 L. Yakhontov, M.Rubtsov, Zhurn. Obshchei Khim., 1958, 28, 1962, 3139; 1959, 29, 1172.

14 H. Shyder, F. Verber, J. Amer. Chem. Soc., 1950, 72, 2962

15 I. Kompish, E. Crossmann, I.Terentjeva, G.Lazurjevski, Khim.Prir.Soedin., 1969, 34.

16 K. Blaha, Z. Koblicova, J. Pospisek, J.Trojanek, Collection, 1971, 10, 3448.

17 T. Shirshova, I. Terentjeva, P. Vember, G. Lazurjevski, Khim.Geterotsikl. Soedin., 1972, 987.

18 T. Shirshova, I. Terentjeva, G. Lazurjevski, V.Adanin, Khim.Geterotsikl. Soedin., 1974, 1133.

19 W. Müller, R. Preup, E. Winterfeldt, Angew. Chemie, 1975, 87, 385.

20 A. Semenov, I. Terentjeva, Khim. Geterotsikl. Soedin., 1965, 235.

21 K. Kuchkova, A. Semenov, I. Terentjeva, Khim. Geterotsikl. Soedin., 1970, 197. Acta Chim. Acad. Sci. Hung., 1971, 69, 367.

22 J. Powers. J. Org. Chem., 1965, 30, 2534.

23 I. Sharipov, N. Cheban, M. Filippov, I. Terentjeva, Izv. Akad. Nauk MSSR, Ser. Biol., Khim. 1975, 1, 75.

24 G. Lazurjevski, I.Terentjeva, "Alkaloids and Plants", Ed. Shtiintsa, Kishinev, 1975.

25 G. Skryabin, L. Golovleva, I. Terentjeva, G. Lazurjevski M. Nefedova, V. Adanin, G. Shmotina, G. Sadyrina. Izv. Akad. Nauk SSSR, Ser. Biol., 1974, 682.

26 K. Mothes, H. Schütte, "Biosynthese der Alkaloide", VEB Berlin, 1969.

27 G. Marku. Sdravoohranenie, Kishinev, 1964, 2, 28.

28 M.Yoshida. Chem. Commun., 1969, 1223.

Received, 21st August, 1976