UDC 547-944

BIOSYNTHESIS OF BREVICOLLINE

I. Kompiš, E. Grossmann, I. V. Terent'eva, and G. V. Lazur'evskii

Khimiya Prirodnykh Soedinenii, Vol. 5, No. 1, pp. 39-43, 1969

In view of the peculiar structure of brevicolline—1-methyl-4-(N-methyl-2-pyrrolidyl)-β-carboline (I) [1, 2], it was of interest to study the mechanism of its biosynthesis.

Table 1

Results of Experiments on the Introduction of Radioactive Preparations into Carex brevicollis

Preparation	Amount		Dilution*	Number of plants	Amount of brevicolline isolated, mg	Specific activity, counts/min/mmole · 10^5	Specific incorporation**, %
	μCi	mg					
DL-[2-^{14}C]tryptophan	0.25	1.30	13.6	12	20	6.30	0,010
sodium [2-^{14}C]pyrotartrate	0.50	20.20	6.5	20	52.8	6.79	0.017
L-[^{14}C]glutamic acid	0.50	1.15	21.8	14	12	0.824	0.0009
sodium [^{14}C]-formate	0.50	4.00	23.9	23	26	0.594	0.012

* The dilution was defined as $\frac{\text{amount of brevicolline after dilution, mg}}{\text{amount of active brevicolline isolated}}$.

** The specific incorporation is defined as $\frac{\text{number of counts in the brevicolline isolated}}{\text{total number of counts introduced into the plant}}$.

Well-known ideas [3, 4] give grounds for assuming that the β-carboline skeleton arises by the condensation of tryptophan (tryptamine) with acetaldehyde or its biological equivalent—pyrotartaric acid. The pyrrolidine grouping, by analogy with nicotine, which has the same structural fragment, can be formed from glutamic acid, and the N-methyl group from formic acid.

Table 2

Absolute and Relative Activities of the Compounds Obtained

Preparation introduced	Compounds obtained	Activity	
		counts/min/ mM · 10^5	% with respect to the activity of the brevicolline
DL-[2-^{14}C]tryptophan	Brevicolline*	6.30	100
	Harmane-4-carboxylic acid	6.17	98
	Harmane**	5.80	92
	Barium carbonate	0	0
[2-^{14}C]pyrotartaric acid	Brevicolline*	6.78	100
	Acetic acid***	6.44	95
	Barium carbonate	5.92	87.3
	N-Methylbenzamide	0.13	1.9
L-[U-^{14}C]glutamic acid	Brevicolline*	0.82	100
	Harmane-4-carboxylic acid**	0.51	61.4
	Harmane**	0.42	50.7
Sodium [^{14}C]-formate	Brevicolline*	0.59	100
	Trimethylammonium iodide	0.52	87

* Measured in a toluene scintillator; ** in a dimethylsulfoxide—dioxane scintillator (1 : 9); *** in the form of α-naphthylacetamide.

To confirm the above, experiments with labelled substances have been carried out. From the plant Carex brevicollis DC grown in a nutrient solution containing DL-[2-^{14}C] tryptophan, radioactive brevicolline with a specific incorporation of 0.01% was isolated (Table 1). Oxidation of the latter gave harmane-4-carboxylic acid (II) containing practically all the activity of the brevicolline. The harmane (III) formed by the decarboxylation of the acid, had 92% of the activ-

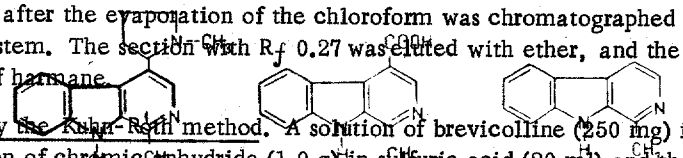
[illegible] ...dation [illegible] ...reliabil- [illegible]

The low activity of the substances in the experiments is due to the fact that the labeled materials entered through the roots. Sodium [2-^{14}C] pyrotartrate also proved to be a source of active brevicolline. In the distillate after its oxida- tion by the Kuhn–Roth method [illegible] contained [illegible] of the activity [illegible] hydroxide [illegible]

These experimental results confirm that in the case of Carex brevicollis DC. the β-carboline skeleton arises from tryptophan and pyrotartaric acid.

Hahn et al. have shown [8] that tryptamine, by condensing with enolized α-keto acids, forms tetrahydro-β-carboline-1-carboxylic acids and only with aldehydes do 1-monosubstituted β-carbolines arise. The formation of active brevicolline in experiments with the introduction of sodium [2-^{14}C] pyrotartrate into the plant permits the assumption that the [illegible] before its condensation with tryptophan (tryptamine).

When the plant was grown on a nutrient solution containing labeled sodium formate, we again obtained radioactive brevicolline with a specific [illegible] 0.012% [illegible] 10 [illegible] of the N-methyl group by the Herzig–Meyer method [7] [illegible] (87%) [illegible]



Thus, the part of the brevicolline molecule (I) shown by heavy lines (formula) arises from tryptophan. Carbon atoms [illegible] pyrotartaric acid. The precursor of the N-methyl group is sodium formate.

When universally labeled glutamic acid [illegible] brevicolline with a low and nonspecific [illegible] (0.007%) was obtained. This does not permit an unambiguous conclusion that this acid is a precursor of the pyrrolidine ring in brevicolline.

Conclusions

1. When DL-[2-^{14}C] tryptophan, sodium [2-^{14}C] pyrotartrate, sodium [^{14}C]-formate, and universally labeled L-[^{14}C] glutamic acid were introduced through the root system into Carex brevicollis DC, active brevicolline was obtained.
2. Tryptophan and sodium pyrotartrate are precursors of the β-carboline moiety of the brevicolline molecule, and sodium formate is a precursor of the N-methyl group.
3. The introduction of universally labeled glutamic acid does not lead to an unambiguous indication of the role of this precursor in the biosynthesis of brevicolline.

Experimental

[illegible] UR-10 spectrophotometer [illegible] containing 0.5–2.5% of [illegible] (PPO) and 0.03% of 1,4-bis(5-phenyl-2-oxazolyl)benzene (POPOP), and 2) in a mixture of dioxane and [illegible] (9:1) [illegible] 10% of [illegible] 0.7% of PPO, and 0.05% of POPOP.

Chromatography was carried out in a thin layer with alumina (neutral, activity grade II), and in a fixed layer with alumina Woelm (neutral).

Introduction of the active precursors and extraction of brevicolline. After the cutting of the roots, plants of Carex brevicollis DC collected in the Kishinev region were grown by the hydroponic method in a solution containing 0.8 g of calcium nitrate, 0.2 g of monopotassium phthalate, 0.2 g of magnesium sulfate, 0.2 g of potassium nitrate, 0.1 g of potassium chloride, and 0.01 g of ferric chloride in 1000 ml of water for 7 days. The roots of the well-developed plants were again shortened by 1–2 cm and the radioactive preparation was added to the solution (in 1 ml of water for one plant).

After 24 hr, the [illegible] the plant was grown [illegible] it for another six days. Then the plant was washed with distilled water, [illegible] 150 ml [illegible] 50 ml of chloroform, and extracted in a mixer for 2 min. The suspension was left for 24 hr and the chloroform was separated off. This treatment was repeated twice more. [illegible] 20 ml of benzene, and the alkaloids were extracted with 0.5% of hydrochloric acid (5 · 20 ml). The acid extract was made alkaline with ammonia and extracted with ether (5 · 20 ml). The ether was driven off and the residue was chromatographed on a layer of alumina in the benzene–methanol (98 : 2) system. After the spots had been revealed with

iodine vapor, in an individual sample the band with R_f 0.19 was cut out and eluted with ether. The concentration of brevicolline in an aliquot of the residue after the evaporation of the ether was determined from the extinction in the UV region of the spectrum. Chromatographically pure brevicolline was diluted with a known amount of an inactive sample. The mp, UV and IR spectra, and chromatograms of the brevicolline obtained agreed with those for an authentic sample (the identity of all the compounds obtained was established in a similar manner).

Oxidation of brevicolline. Brevicolline (100 mg) was oxidized with chromic acid as described previously [1b], with the only difference that the precipitate of barium chromate was separated by centrifuging and the solution after the precipitation of the Ba^{++} in the form of the carbonate (reagent-grade ammonium carbonate) and filtration was evaporated in vacuum to 10 ml. Acidification of the solution with sulfuric acid gave a yellow precipitate of harmane-4-carboxylic acid (10.9 mg) (II). The esterification of (II) with methanol in the presence of sulfuric acid formed methyl harmane-4-carboxylate.

Decarboxylation of harmane-4-carboxylic acid (II). A suspension of 26.5 mg of (II) and 300 mg of fine copper bronze in 3 ml of paraffin oil was heated at 250° C in a current of nitrogen for 5 min.

The carbon dioxide was trapped with a saturated solution of barium hydroxide. The barium carbonate (14.1 mg, 61%) was filtered off and washed with water, ethanol, and ether.

After the reaction, the suspension was cooled, diluted with 10 ml of ether, and filtered. The filtrate was extracted with 1% hydrochloric acid, and the aqueous solution was made alkaline with ammonia and extracted with ether. The ethereal solution was again treated with hydrochloric acid and the alkaloids from the aqueous solution were extracted with chloroform. The residue after the evaporation of the chloroform was chromatographed in a layer of alumina in the benzene–methanol (97 :3) system. The section with R_f 0.27 was eluted with ether, and the solvent was evaporated off. This gave 21.4 mg (62.5%) of harmane.

Oxidation of brevicolline by the Kuhn–Roth method. A solution of brevicolline (250 mg) in 10 ml of 2 N sulfuric acid was added to a stirred solution of chromic anhydride (1.0 g) in sulfuric acid (20 ml) and the mixture was heated for 2 hr. Then distillation with steam in a current of nitrogen gave 100 ml of a distillate which was neutralized with 0.5 N caustic soda and evaporated to dryness (30 mg). The presence of sodium acetate was established by paper chromatography [9]. For measuring its activity, the sodium acetate was converted into the α-naphthylamine derivative [5].

Degradation of sodium acetate by the Schmidt reaction [7]. The reaction was carried out with 25 mg of active sodium acetate in 1 ml of concentrated sulfuric acid and 50 ml of sodium azide.

The carbon dioxide was isolated in the form of barium carbonate (75 mg) and the methylamine in the form of N-methylbenzamide (10.0 mg) (Table 2).

REFERENCES

1. a) I. V. Terent'eva, in the collection: Alkaloid-Bearing Plants of Moldavia [in Russian], Kishinev, p. 21, 1960; b) P. A. Bember, I. V. Terent'eva, and G. V. Lazur'evskii, KhPS [Chemistry of Natural Compounds], 3, 249, 1967.
2. V. M. Chernov, in the collection: Alkaloid-Bearing Plants of Moldavia [in Russian], Kishinev, p. 49, 1960; M. D. Mashkovskii, Medicinal Substances [in Russian], Moscow, p. 88, 1964.
3. K. Mothes and H. R. Schutte, Angew. Chemie, 75, 370, 1963.
4. E. Leete, J. Amer. Chem. Soc., 82, 6388, 1960.
5. E. Leete, A. Ahmad, and I. Kompis, J. Amer. Chem. Soc., 87, 4168, 1965.
6. H. R. Schutte, Radioaktive Isotope in der organischen Chemie und Biochemie, Deutsche Verlag d. Wissenschaften, Berlin, 154, 1966.
7. H. R. Schutte, Radioaktive Isotope in der organischen Chemie und Biochemie, Deutsche Verlag d. Wissenschaften, Berlin, 183, 1966.

iodine vapor, in an individual sample the band with R_f 0.19 was cut out and eluted with ether. The concentration of brevicolline in an aliquot of the residue after the evaporation of the ether was determined from the extinction in the UV region of the spectrum. Chromatographically pure brevicolline was diluted with a known amount of an inactive sample. The mp, UV and IR spectra, and chromatograms of the brevicolline obtained agreed with those for an authentic sample (the identity of all the compounds obtained was established in a similar manner).

Oxidation of brevicolline. Brevicolline (100 mg) was oxidized with chromic acid as described previously [1b], with the only difference that the precipitate of barium chromates was separated by centrifuging and the solution after the precipitation of the Ba^{++} in the form of carbonate (reagent—ammonium carbonate) and filtration was evaporated in vacuum to 10 ml. Acidification of the solution with sulfuric acid gave a yellow precipitate of harmane-4-carboxylic acid (10.9 mg) (II). The esterification of (II) with methanol in the presence of sulfuric acid formed methyl harmane-4-carboxylate.

Decarboxylation of harmane-4-carboxylic acid (II). A suspension of 26.5 mg of (II) and 300 mg of fine copper bronze in 3 ml of paraffin oil was heated at 250° C in a current of nitrogen for 5 min.

The carbon dioxide was trapped with a saturated solution of barium hydroxide. The barium carbonate (14.1 mg, 61%) was filtered off and washed with water, ethanol, and ether.

After the reaction, the suspension was cooled, diluted with 10 ml of ether, and filtered. The filtrate was extracted with 1% hydrochloric acid, and the aqueous acid solution was made alkaline with ammonia and extracted with ether. The ethereal solution was again treated with hydrochloric acid and the alkaloids from the aqueous solution were extracted with chloroform. The residue after the evaporation of the chloroform was chromatographed in a layer of alumina in the benzene—methanol (97 :3) system. The section with R_f 0.27 was eluted with ether, and the solvent was evaporated off. This gave 21.4 mg (62.5%) of harmane.

Oxidation of brevicolline by the Kuhn-Roth method. A solution of brevicolline (250 mg) in 10 ml of 2 N sulfuric acid was added to a stirred solution of chromic anhydride (1.0 g) in sulfuric acid (20 ml) and the mixture was heated for 2 hr. Then distillation with steam in a current of nitrogen gave 100 ml of a distillate which was neutralized with 0.5 N caustic soda and evaporated to dryness (30 mg). The presence of sodium acetate was established by paper chromatography [9]. For measuring its activity, the sodium acetate was converted into the α-naphthylamine derivative [5].

Degradation of sodium acetate by the Schmidt reaction [7]. The reaction was carried out with 25 mg of active sodium acetate in 1 ml of concentrated sulfuric acid and 50 ml of sodium azide.

The carbon dioxide was isolated in the form of barium carbonate (75 mg) and the methylamine in the form of N-methylbenzamide [10] (Table 2).

Conclusions

1. When DL-[2-^{14}C] tryptophan, sodium [2-^{14}C] pyrotartrate, sodium [^{14}C]-formate, and universally labeled L-[^{14}C] glutamic acid were introduced through the root system into Carex brevicollis DC, active brevicolline was obtained.
2. Tryptophan and sodium pyrotartrate are precursors of the β-carboline moiety of the brevicolline molecule, and sodium formate is a precursor of the N-methyl grouping.
3. The introduction of universally labeled L-glutamic acid does not lead to an unambiguous indication of the role of this precursor in the biosynthesis of brevicolline.

REFERENCES

1. a) I. V. Terent'eva, in the collection: Alkaloid-Bearing Plants of Moldavia [in Russian], Kishinev, p. 21, 1960; b) P. A. Bember, I. V. Terent'eva, and G. V. Lazur'evskii, KhPS [Chemistry of Natural Compounds], 3, 249, 1967.
2. V. M. Chernov, in the collection: Alkaloid-Bearing Plants of Moldavia [in Russian], Kishinev, p. 49, 1960; M. D. Mashkovskii, Medicinal Substances [in Russian], Moscow, p. 88, 1964.
3. K. Mothes and H. R. Schutte, Angew. Chemie, 75, 370, 1963.
4. E. Leete, J. Amer. Chem. Soc., 82, 6388, 1960.
5. E. Leete, A. Ahmad, and I. Kompiš, J. Amer. Chem. Soc., 87, 4168, 1963.
6. H. R. Schutte, Radioaktive Isotope in der organischen Chemie und Biochemie, Deutsche Verlag d. Wissenschaften, Berlin, 154, 1966.
7. H. R. Schutte, Radioaktive Isotope in der organischen Chemie und Biochemie, Deutsche Verlag d. Wissenschaften, Berlin, 183, 1966.

8. G. Hahn, L. Barwald, O. Schales, and H. Werner, Ann. 520, 107, 1935; G. Hahn and F. Rumpf, Ber., 71, 2141, 1938.
9. E. P. Kennedy and H. A. Barker, Anal. Chem., 23, 1033, 1951.
10. E. Leete, H. Gregory, and E. G. Gross, J. Amer. Chem. Soc., 87, 3475, 1965.

4 December 1967

Institute of Chemistry, Slovak AS
Institute of Chemistry, AS MoldavSSR