Tetrahedron Vol. 24, pp 1323 to 1326 Pergamon Press 1968. Printed in Great Britain

THE STRUCTURE OF GENTIOFLAVINE, A NEW ALKALOID OF SOME GENTIANA SPECIES

N. L. MAREKOV and S. S. POPOV

Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

(*Received in UK* 30 *March* 1967; *accepted for publication* 12 *July* 1967)

Abstract—Gentioflavine, a new alkaloid from some Gentiana species, is shown to be a dihydropyridine lactonic alkaloid. Its structure (III) has been established on the basis of NMR and IR spectral evidence, oxidative degradation and conversion into the known alkaloid gentianidine (II).

THE alkaloid IV, now renamed gentioflavine, has been isolated from some Gentiana species.[1] It crystallizes as yellow prisms, m.p. 218–220° (dec) and is almost insoluble in hexane and benzene, sparingly soluble in chloroform, and more soluble in alcohol, pyridine and water.

Preliminary studies show that the carbon skeleton of gentioflavine differs from that of gentianine, the main alkaloid of Gentiana species, and that it is not converted into gentianine or any gentianine derivative. Instead, a mixture of many products was always produced the identification of which was impossible owing to the small amounts obtained. In most cases the IR spectrum indicated the presence of aromatic compounds, whereas gentioflavine itself does not possess an aromatic ring.

Elemental analysis and mol wt determination by mass spectrometry indicated $C_{10}H_{11}NO_3$ as the molecular formula for gentioflavine. It is weakly basic, does not form salts with acids, gives a negative reaction with Dragendorff's reagent, but a positive one with Mayer's reagent, dilute mineral acids yield a mixture of polar products, while resinification occurs with alkalis.

The UV spectrum of gentioflavine has maxima at 235, 298 and 410 nm. The IR spectrum (in Nujol) shows the presence of an —NH— group (3235 cm^{-1}), a conjugated lactone group (1700 cm^{-1}), a conjugated carbonyl group (1640 cm^{-1}) and a conjugated double bond (1620 cm^{-1}); a maximum for an aromatic (pyridine) ring is absent. Its NMR spectrum (in pyridine) shows 7 groups of peaks: singlets at 10·1 δ, 8·45 δ and 8·8 δ, doublet at 1·3 δ, triplets at 3 δ and 4·35 δ, and quadruplet at 5·2 δ with ratios of the intensities approximately as follows 1:1:1:3:2:2:1. The doublet at 1·3 δ and the quadruplet at 5·2 δ are attributed to the group CH—CH_3, the two triplets to —CH_2—CH_2—, adjacent to a quaternary carbon with an electronegative atom (oxygen) at either end; the singlet at 10·1 δ could be attributed to a proton of an aldehyde or carboxylic group, attached to a C devoid of H-atoms. The other two singlets could be due to a NH-proton and to a proton, bonded with an unsaturated carbon forming a strong hydrogen bond.

The presence of the aldehyde group was confirmed by the test with ammoniacal silver nitrate solution, the formation of a semicarbazone, m.p. 221–223° (dec) and an oxime, m.p. 203–205° (dec).

Valuable data on the structure of gentioflavine was obtained by oxidation (1) with concentrated nitric acid which produced 2·5 moles of carbon dioxide and yielded as main product 3,4,5-pyridine tricarboxylic acid and a certain amount of 4-hydroxy-3,5-pyridine dicarboxylic acid; and (2) with cold bromine water, which produced hydrogen bromide and 1 mole carbon dioxide, and a bromine-containing product—bromogentioflavine, m.p. 133–135°. Bromogentioflavine being basic, formed a picrate, m.p. 174–176° (dec). The molecular formula $C_9H_8O_2NBR$ was assigned to bromogentioflavine on the basis of elemental analysis of its picrate and mol wt determination by mass spectrometry. Its IR spectrum in chloroform shows the presence of lactonic carbonyl (1740 cm^{-1}), a Me group (2860 cm^{-1}) and an aromatic ring (1590 cm^{-1}); the max for the NH-group as well as for a conjugated carbonyl group and a double bond, typical of the gentioflavine IR spectrum, are absent.

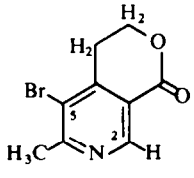
The NMR spectrum of bromogentioflavine (in $CDCl_3$) has four groups of peaks: singlets at 2·76 δ and 9·03 δ, and triplets at 3·16 δ and 4·57 δ, having a ratio of intensities approximately 3:1:2:2. The singlet at 2·76 δ indicates the presence of a Me group, adjacent to a carbon atom devoid of H-atoms; the singlet at 9·03 δ could be an aromatic proton forming a strong hydrogen bond; the two triplets corresponded to methylene groups, similar to those of gentioflavine.

The presence of a lactone ring in bromogentioflavine was shown by treatment with alcoholic sodium hydroxide. The strongly polar amorphous product obtained, regenerated bromogentioflavine, when reacidified.

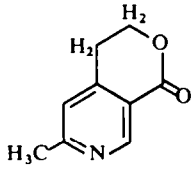
These reactions are in keeping with structure I. Of the two possible positions for the bromine atom (2 and 5), position 5 is preferable and in accordance with the data of the NMR spectrum of bromogentioflavine in which the singlet at 9·03 δ could be attributed to a proton in a pyridine ring forming a hydrogen bond with the lactonic carbonyl.

Finally, structure I for bromogentioflavine was confirmed by its transformation into the alkaloid gentianidine (II). The latter was recently found in *Gentiana macrophylla* and its structure established and confirmed by synthesis.[2] The removal of bromine from bromogentioflavine was achieved by treatment with Raney nickel,[3] and the product, m.p. 126–128° (benzene–hexane) was identified chromatographically as gentianidine. Mixed with an authentic sample it showed no depression in m.p.

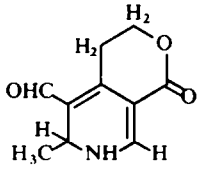
Consequently structure III can be assigned to the alkaloid gentioflavine.



I



II



III

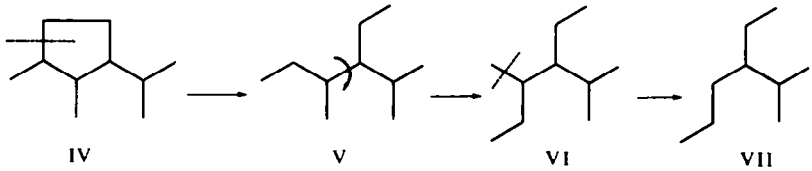
When gentioflavine was oxidized with conc nitric acid, the 3,4,5,6-pyridine tetracarboxylic acid formed was converted under the reaction conditions into 3,4,5-pyridine tricarboxylic acid, and by means of further decarboxylation and oxidation at the 4-position, 4-hydroxy-3,5-pyridine dicarboxylic acid was obtained.

The interaction of gentioflavine with bromine water results in oxidation of the aldehyde group to a carboxyl and decarboxylation under the action of bromine water. Simultaneously, the dihydropyridine ring is oxidized to pyridine.

Biogenesis. Pyruvate and formate do not participate in the formation of the gentianine molecule[4] which does not support the tyrosine,[5] prephenate[6] and acetate[7] hypothesis and is in agreement with the terpene[8] hypothesis for the formation of the carbon skeleton of gentianine and of the non-tryptophan portion of indole alkaloids. After administration of [1 – ^{14}C] pyruvate and [^{14}C] formate to *Gentiana asclepiadea* radioinactive gentioflavine was isolated. It is quite possible that both gentianine and gentioflavine could have a terpene origin.

The carbon skeleton of gentioflavine is very close to that of erythrocentaurine, isolated from *Erythraea centaurium* and recently from *Gentiana lutea.*[9] Kubota and Tomita[10] assumed that erythrocentaurine was a secondary product obtained by hydrolysis of swertiamarine. We established experimentally that gentioflavine could be obtained from Gentiana species (the experiments were carried out with roots of *Gentiana lutea*) either by treatment of the methanol extracts with hydrochloric acid and ammonia or without the use of hydrochloric acid and treatment with excess sodium carbonate. We showed, however, that gentioflavine could be isolated from *Erythraea centaurium* only in the case when ammonia has been used to make the extract alkaline.[11]

Gentianidine possesses only nine carbon atoms which presents difficulties in the postulation of a terpene biogenesis for this alkaloid. The solution of this problem was facilitated by the establishment of the structure of gentioflavine. A comparative examination of the carbon skeletons in gentianine, gentioflavine and gentianidine indicated a close biogenetic relationship as shown in the scheme:



IV V VI VII

The formation of these three carbon skeletons could be explained on the basis of a common terpenoid precursor IV, suggested by Thomas[8] as a precursor of this and of certain indole and other alkaloids. By cleavage of a carbon bond in this precursor the gentianine skeleton V is obtained from which the gentioflavine skeleton VI may be obtained if one part of the molecule is turned through 180°. The gentianidine skeleton VII is obtained from the gentioflavine skeleton VI by elimination of a C_1 unit. Thus gentioflavine appears to be a missing link needed to explain the formation of gentianidine.

Recently, gentianine, gentioflavine and gentianidine were found in *Erythraea centaurium,*[11] which supports the close biogenetic relationship between these alkaloids.

EXPERIMENTAL

All m.ps are uncorrected. IR spectra were determined on an UR-10 spectrometer; NMR spectra were determined on a Varian A-60 spectrometer with TMS as internal standard.

Oxidation of gentioflavine with conc nitric acid. A mixture of gentioflavine (150 mg) and HNO_3 (3 ml; d = 1·36) was heated for 4 hr on an oil bath, the temp rising to 100–140°; the CO_2 formed was absorbed in a 5% $Ba(OH)_2$ aq. The excess HNO_3 was removed under reduced press to dryness several times and finally the solution was concentrated to 3 ml and cooled to yield 25 mg of a colourless crystalline product which after recrystallization from water had m.p. 327–330° (dec). It gave IR spectrum identical with that

of 4-hydroxy-3,5-pyridinedicarboxylic acid. Under slow concentration of the mother liquor another crystalline product (14 mg) was obtained which after recrystallization from water had m.p. 258–260° (dec.) and IR spectrum is identical with that of 3,4,5-pyridinetricarboxylic acid.

70·4 mg CO_2 were obtained on oxidation.

Oxidation of gentioflavine with bromine water. Bromine water was added dropwise slowly to a saturated aqueous solution of gentioflavine (200 mg) until the yellow colour of the latter disappeared and a colourless ppt was formed; the CO_2 produced was absorbed in a 5% $Ba(OH)_2$ aq. The reaction mixture was made alkaline with ammonia and extracted 4 times with ether. The ether extracts were combined and evaporated to dryness the residue being recrystallized from MeOH to yield 140 mg of a colourless crystalline product, m.p. 133–135° which formed a picrate, m.p. 174–176° (dec). (Found: C, 38·51; H, 2·65. Calc for $C_{13}H_{11}O_9N_4Br$: C, 38·22; H, 2·33%.)

30·8 mg CO_2 were formed from this oxidation.

Opening of the lactone ring in bromogentioflavin. NaOH (20 mg) dissolved in EtOH (2 ml) was added to a saturated EtOH soln containing 60 mg bromogentioflavine. The mixture was heated to 50° for 15 min, cooled and ether (100 ml) added. A white ppt of the Na-salt of the hydroxy acid was obtained which was separated after 12 hr and washed with ether and chloroform until a negative Dragendorff's reaction resulted. It was acidified with HCl (1:4) and after 12 hr was made alkaline with ammonia and then extracted with ether. After evaporation the ether extracts gave 30 mg of a colourless crystalline product with m.p. not depressed by admixture with the original sample of bromogentioflavine.

Debromination of bromogentioflavine. The Raney Ni catalyst was obtained by heating Ni-Al (3 g) with 20% KOH aq (15 ml) for 1 hr at 50° and washing with water until pH 7–8 and then with MeOH. The catalyst was suspended in MeOH (20 ml) and then 1N KOH (18 ml) in bromogentioflavine (200 mg) was added. The mixture was stirred for 80 min (for 45 min period the debromination was partial), the catalyst filtered and washed with MeOH and water; the filtrates were collected, the solvent evaporated, the residue acidified, made alkaline with ammonia and extracted with ether to yield colourless needles, m.p. 126–128° (benzene–hexane) and no m.p. depression when mixed with an authentic sample of gentianidine.

Semicarbazone of gentioflavine. Gentioflavin (100 mg), dissolved in water, was mixed with a sat AcONa aq (150 mg) and semicarbazide hydrochloride (100 mg). After heating for 15 min at 100° and cooling yellow crystals of the semicarbazone were precipitated; recrystallization from water gave m.p. 221–223° (dec).

Oxime of gentioflavine. Gentioflavine (100 mg) and hydroxylamine (100 mg) were dissolved in a 2 ml mixture of pyridine–EtOH (1:1). The solvent was then removed and the residue washed with cold water (1 ml) and recrystallized from 70% EtOH to yield yellow crystals, m.p. 203–205° (dec).

Acknowledgement—We wish to thank Mr. St. Spassov, Institute of Organic Chemistry, Bulgarian Academy of Sciences, for NMR spectra and helpful discussion; Dr. L. Dolejs and Dr. V. Hanus, Institute of Physical Chemistry, Czechoslovak Academy of Sciences, Prague, for mass spectral determination of the mol wts.

REFERENCES

[1] N. Mollov, N. Marekov, S. Popov and B. Kouzmanov, *Compt. rend. Acad. bulg. Sci.* **18**, 947 (1965).
[2] Liang Xiao-tian, Yu De-quan and Fu Feng-yung, *Scientia Sinica*, **14**, 869 (1965).
[3] H. Kämmerer, L. Horner and A. Beck, *Ber.*, **91**, 1377 (1958).
[4] N. Marekov, S. Popov and G. Georgiev, *Compt. rend. Acad. Bulg. Sci.*, **19**, 827 (1966).
[5] T. Govindachari, K. Nagarajan, S. Rajappa, *Experientia*, **14**, 5 (1958).
[6] E. Wenkert, N. Bringi, *J. Am. Chem. Soc.*, **81**, 1474 (1959).
[7] E. Leete, S. Ghosal, *Tetrahedron Letters*, 1179 (1962).
[8] R. Thomas, *Tetrahedron Letters*, 544 (1961).
[9] N. Marekov, N. Mollov and S. Popov, *Compt. rend. Acad. Bulg. Sci.*, **18**, 999 (1965).
[10] T. Kubota and Y. Tomita, *Tetrahedron Letters*, 176 (1961).
[11] N. Marekov, S. Popov, *Compt. rend. Acad. Bulg. Sci.* (in press).