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CHEMICAL CONSTITUENTS OF GENTIANACEAE. VIII*
THE STRUCTURE OF GENTIOCRUCINE, A NOVEL LACTONIC ENAMINO KETONE

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Gentiocrucine was first isolated from Gentiana cruciata by Popov and Marekov[1], who proposed structure (I) for the compound on the basis of the following facts: (i) Molecular formula, $C_6H_7O_3N$ (elemental analysis and M^+); (ii) IR absorption spectrum ($CHCl_3$): 3480 cm^{-1} (NH_2), 1705 cm^{-1} (conjugated lactonic carbonyl), 1642 cm^{-1} (conjugated amide), 1622 cm^{-1} (conjugated double bond); (iii) PMR spectrum (D_2O): a pair of two-proton triplets at 2.58 and 4.40 ppm ($-CH_2-CH_2-$), a one-proton broad signal at 8.1 ppm (deshielded olefinic proton); (iv) MS: m/e 141 (M^+), fragment ion peaks at m/e 113, 111, 97, 83 (due to elimination of CO, CH_2O, CO_2, and $CO+CH_2O$ from the molecular ion); (v) decolourization of bromine water; (vi) formation of ammonium chloride upon hydrolysis with dilute HCl (the non-nitrogenous acidic part was not characterized.

We have isolated the same compound[2] from another plant, Enicostemma hyssopifolium (Willd.) Verd. (syn. E. littorale Blume) and reinvestigated its structure for the following reasons. During staining of the carbonyl components present in the alkaloid fraction of the plant extract, gentiocrucine showed a yellowish-brown spot with 2:4-dinitrophenylhydrazine reagent on TLC plates. Subsequently, upon graded concentration of the reaction mixture, it yielded two dinitrophenylhydrazones (DNPH), (A) $C_{12}H_{11}O_6N_5$, orange needles, m.p. 214-216°, γ_{max} (EtOH) 255, 318, 408 nm and (B)

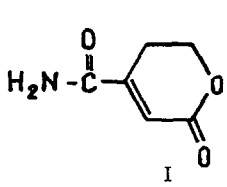
*Part VII: S. Ghosal, R.K. Chaudhuri and A. Nath, Phytochemistry, 12, 1763 (1973).

$C_{12}H_{10}O_7N_4$, bright red needles, m.p. 194-195°, γ_{max} (EtOH) 258, 315, 405 nm. The UV spectra of the derivatives are reminiscent of those of cross conjugated dienone chromophores[3]. Gentiocrucine itself showed UV absorption, γ_{max} (EtOH) 232 (log ε, 4.35), 283 nm (4.37); γ_{max} ($HClO_4$) 230, 273 nm; γ_{max} (EtOH-0.1N KOH) 240, 275 nm, attributable to the presence of an enamino ketone[4] having a cross conjugated lactone function[5]. Gradual decomposition of the chromophoric systems was observed both in acid and alkali media. The IR spectrum (in KBr) exhibited strong bands at 3400 and 3260 cm^{-1} which are shifted by about 100 and 140 cm^{-1} from the normal positions of a free NH_2 group. The phenomenon could be attributed to the participation of the amino protons in unequal hydrogen bonding[6]. The appearance of the other major bands, with their possible assignments in parentheses, 1705 cm^{-1} (broad, hydrogen bonded $\alpha\beta$-unsaturated lactone carbonyl), 1625 cm^{-1} (broad, hydrogen bonded carbonyl as in $\alpha\beta$-unsaturated β-amino ketone[7]), 1495 cm^{-1} (C=C coupled with CO and C-N), 842 cm^{-1} (trisubstituted double bond), indicated that there could be more than one species contributing to the structure of gentiocrucine. The presence of an aliphatic enamino ketone function in the compound was also indicated by its mass spectral fragmentation which showed, beside the molecular ion peak at m/e 141 (100%), a significant fragment ion peak at m/e 114 (24%, metastable peak observed at m^* 92.5 from 141→114 transition). The loss of 27 m.u. is not likely to be due to loss of C_2H_3 (from the lactone methylenes) as to loss of HCN (from the enamino ketone moiety) as none of the lactonic gentiana alkaloids, investigated[8] so far, exhibited any identifiable peak at M-27. Popov and Marekov quoted[1] only limited mass data (without mentioning the relative abundance of the ions) for gentiocrucine, and the proposed assignments were also not convincing. The molecular ion peak of gentiocrucine, contrary to what one would expect for a δ-lactone[9], constituted the base peak and the fragment ions at m/e 111 and 83, unlike those of a δ-lactone, being only minor peaks (8 and 1%). Also, the proposed[1] retention of the $CONH_2$ group in the fragment ions seems unlikely in view of the fact that this group suffers ready fragmentation into NH_2 and CO on electron impact[9]. The mentioned and other fragment ion peaks at m/e 113 (10), 112 (2), 98 (14), 97 (1), 96 (4), 86 (2), 85 (4), 83 (1), 70 (15), 69 (64), 68 (12), 55 (12), would reveal the complex nature of the molecule[10]. If the premise is granted that there is a function $OC-\dot{C}=CH-NH_2$ in gentiocrucine (formation of two DNPHs from the $\alpha\beta$-unsaturated ketone and the aldehyde function generated by hydrolysis of the enamine in situ, UV spectra of the compound and its derivatives, IR spectrum, loss of HCN from M^+), then the resonance stabilized species (a and b) would be the plausible pictures for the two significant fragment ion peaks at m/e 70 and 69, respectively.

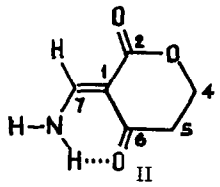
$\overset{+}{O}\equiv C-CH=CH-NH_2$ (a) m/e 70

$\overset{+}{O}\equiv C-\dot{C}=CH-NH_2$ (b) m/e 69

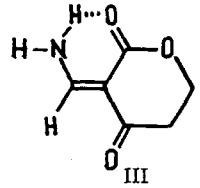
The 100 MHz Fourier transform PMR spectrum of gentiocrucine brought further clarity into its structure. It showed (in DMSO-d_6) two two-proton triplets at 2.4 (partly overlapping with the solvent signal) and 4.3 ppm, due to methylene protons; a one-proton complex multiplet at 8.1 ppm consisting of a superposition of two doublets of doublets originating from cis and trans coupling (J = 9 and 17 Hz) of the olefinic proton, in two environments with the non-equivalent NH_2 protons. The latter appeared as two broad signals of unequal intensity at 9.2 and 10.0 ppm. The complex pattern of the olefinic proton simplified to two singlets of unequal intensities after deuterium exchange. There was an apparent small coupling in the triplet at 4.3 ppm, which, however, is due to an overlap of two triplets belonging to two different species. For the high field triplet the chemical shift difference in the two species are too small to be observable. The two components in solution are therefore cis-trans isomers (II $\rightleftharpoons$ III). These structures could also accommodate the findings of Popov and Marekov, as aliphatic enamines readily hydrolyze with acids to give ammonium salts; and enamines, but not αβ-unsaturated lactones (of the type I), would be expected to be halogenated on titration with bromine water. Definite proof for the enamine structures (II and III) for gentiocrucine was gained from its further chemistry, in particular, its reduction with 98% formic acid[11] to the β-amino ketone, m.p. 68-69°



I



II



III

The 25.2 MHz ^{13}C Fourier transform NMR spectrum of gentiocrucine exhibited chemical shifts and relative intensities from which the following straightforward assignments can be made (Table 1)

Table 1 - ^{13}C NMR Spectral Data of Gentiocrucine

Carbon	δ_C*)	
	II	III
1	96.96	97.40
2	168.69	168.09
4	63.59	63.48
5	36.28	35.70
6	194.05	191.09
7	157.63	159.35

*) ppm rel. to internal $(CH_3)_4Si$

An αβ-unsaturated ketone is known to absorb between 195 and 205 ppm. The two lowest field resonances are therefore assigned to C_6. The chemical shift difference encountered in the carbonyl resonances (at C_6) is due to polarization of the CO bond by hydrogen bonding[12] as in (II). The signal at 194 would therefore belong to the ketone carbonyl carbon in the cis isomer (II) which is also the more abundant species. The chemical shifts of the lines at 168 ppm are characteristic of lactone carbonyls. The high intensity peaks at 159 and 157 must belong to a proton-bearing carbon (C_7). The only remaining sp^2 carbon is C_1 which appears around 97 ppm. The high field signals (at 63 ppm and 35 ppm / 36 ppm) are designated to C_4 and C_5, respectively. Since spin-lattice relaxation times and line widths can be assumed to be same for corresponding carbons in (II) and (III), the molar ratio of the two isomers can be determined from relative peak heights.

Stable aliphatic primary enamino ketones, such as this, have not been encountered before in nature or prepared synthetically.

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10 Dr. Popov recently reported to us the complete MS data of gentiocrucine which are indistinguishable from our data reported in this communication.

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