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IN VITRO TRANSFORMATIONS OF GENTIOPICROSIDE AND SWERTIAMARIN

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Secoiridoid glucosides and monoterpene alkaloids in Gentianaceae plants possess different carbon skeletons, and their biogenetic relationships were clarified only when the alkaloid gentioflavine [**1**] was isolated and its structure elucidated (1). This alkaloid can be produced from gentiopicroside [**2**] and, in its turn, can be transformed into the alkaloid gentianidine [**3**]. These transformations were confirmed by in vivo experiments with appropriately labeled precursors (2,3).

On the other hand, Kubota and coworkers have demonstrated that, when treated consecutively with NH_3 and HCl, gentiopicroside [**2**] (4) and swertiamarin [**4**] (5) produce the monoterpene alkaloid gentianine [**5**]. Floss *et al.* (6) and Marekov *et al.* (7) established that a substantial number of *Gentiana* alkaloids were produced when the plant extracts were treated with NH_3. The remaining alkaloids are native, for they are also found when NH_3 is not used for alkalinization of the extracts. Evidently, at least one stage of the biosynthesis of *Gentiana* alkaloids can be accomplished in vitro. In this work we report the results of a more detailed study of the action of NH_3 on gentiopicroside [**2**] and swertiamarin [**4**].

GENTIOPICROSIDE AND NH_3.—These experiments were carried out under the reaction conditions used by Kubota and Kamikawa (4).

A solution of gentiopicroside in EtOH, saturated with NH_3, was left overnight and then evaporated, refluxed with acid, and alkalinized with NH_3. After extraction and chromatography, three basic substances were isolated in approximately equal amounts. Two of them were identified as gentianine [**5**] and gentianidine [**3**] by comparison (tlc, ms, mmp) with authentic samples. A third substance, which invariably formed a spot at the starting line of the chromatogram, was unidentified.

Gentianine [**5**] has the skeleton of gentiopicroside [**2**], and its formation from the latter requires only a simple structural transformation. Conversely, the formation of gentianidine [**3**] is accomplished by skeleton alteration, and it obviously includes formation of intermediates.

In a search for such intermediates in a separate experiment we analyzed the reaction product of gentiopicroside [**2**] and NH_3, prior to its treatment with HCl. In this case the reaction mixture had a different composition. As in the previous experiment gentianine [**5**] and the unknown polar alkaloid were found, but, instead of gentianidine [**3**], two other alkaloids were isolated. One of them was identified as gentioflavine [**1**] by comparison (tlc, ms, mmp) with an authentic sample. Gentioflavine holds a key position in the proposed biogenetic scheme of *Gentiana* alkaloids (2), and this is the first time that it has been synthetically obtained. The second alkaloid, isolated in a small amount, was readily transformed into gentianine [**5**], especially in acidic medium. Its mass spectrum corresponds to gentianine plus H_2O.

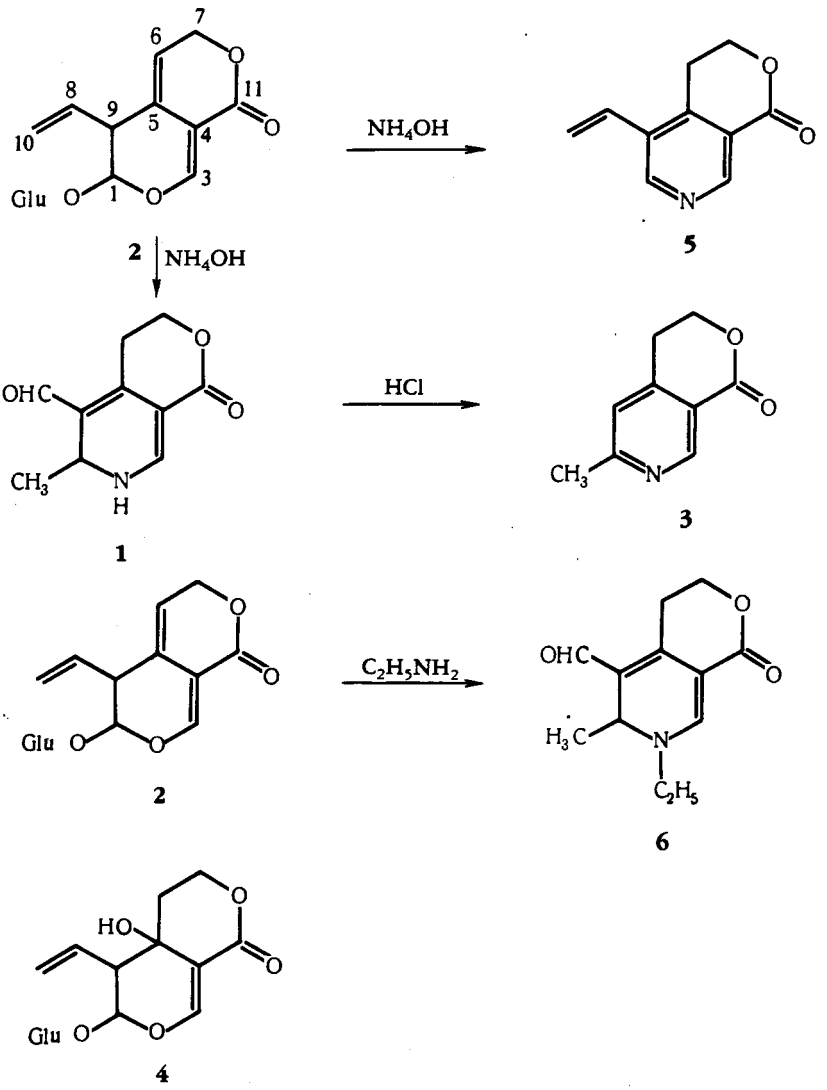
The absence of gentioflavine [**1**] after acidic treatment and the formation of gentianidine [**3**] only after such treatment was an indication that, under these conditions, gentioflavine [**1**] is converted into gentianidine. This assumption was confirmed by the isolation of

gentianidine as the major product of the treatment of gentioflavine with HCl. This transformation includes oxidation of the dihydropyridine ring to a pyridine ring and decarbonylation of the aldehyde group—a process which is probably connected with the amide-like structure of gentioflavine.

The above-mentioned transformations proceed also in H_2O or $EtOH/H_2O$ solutions saturated with NH_3, but in this case the yields are lower. When peracetylated gentiopicroside was used, the same products in similar yields were obtained.

As shown in Scheme 1, the reaction of gentiopicroside with NH_3 can proceed with either the conservation of the gentiopicroside carbon skeleton (gentianine) or rearrangement (gentioflavine). Gentioflavine can be formed after opening of the dihydropyran ring (1,4 addition of NH_3), rotation around the 5,9 bond, conjugation of the 5,6 double bond, and recyclization. The isolation of gentioflavine as a racemate is in accordance with the proposed rearrangement, which involves participation of the vinyl group.

These chemical transformations of



gentiopicroside [**2**] can be regarded as biomimetic reactions providing support to the biogenetic sequences of *Gentiana* iridoids and alkaloids, deduced earlier from structural considerations and incorporation data (2,3).

SWERTIAMARIN [**4**] AND NH_3.—In the experiments, carried out with or without acidic treatment, the main product was always identified as gentianine [**5**], accompanied by more polar alkaloids. We can expect the formation of gentioflavine, but it has not been detected in the reaction mixtures even after mass spectral search. This is an indication that the presence of a 5,6 double bond is important for the rotation around the 5,9 bond, leading to the gentioflavine skeleton.

GENTIOPICROSIDE [**2**] AND ETHYLAMINE.—*N*-alkylated compounds, common in other classes of alkaloids, have never been found in Gentianaceae plants. The conversion of gentiopicroside [**2**] into monoterpene alkaloids under the action of NH_3 provides a possibility for the synthesis of *N*-alkyl monoterpene alkaloids.

When gentiopicroside was treated with ethylamine overnight, the tlc spot of the main product was similar to gentioflavine [**1**] in color and fluorescence, but it was less polar. The molecular ion (*m/z* 221) corresponded to *N*-ethylgentioflavine [**6**]. The elimination of a methyl group produces an ion with *m/z* 206 (base peak). The $[M]^+/[M-15]^+$ ratio is close to this in the gentioflavine mass spectrum, which is an indication for the presence of a 1,2-dihydropyridine ring and a methyl group at C-1 in **6**. Further elimination of ethylene occurred, probably from the *N*-ethyl group, which led to an ion identical to the $[M-15]^+$ ion in the mass spectrum of gentioflavine. This was confirmed by the identity of the further fragmentation of both alkaloids.

The uv spectrum (EtOH) of *N*-ethylgentioflavine (238, 297, and 415 nm) is almost identical with that of gentioflavine (235, 298, and 410 nm).

The ^1H nmr spectrum of *N*-ethylgentioflavine in $CDCl_3$ is similar to that of gentioflavine [**1**]. Two two-proton triplets at δ 3.03 and 4.4 are attributed to the methylene groups of the lactone ring. A one-proton sharp singlet at δ 9.68 could be attributed to the aldehyde proton. The one-proton singlet at δ 7.86 is due to H-3. The three-proton doublet at δ 1.20 ($J = 7$ Hz) and the one-proton quartet at 4.8 ($J = 7$ Hz) are due to the CH-Me group, and the three-proton triplet at δ 1.35 and the two-proton multiplet at δ 3.5 could be attributed to the *N*-ethyl group.

EXPERIMENTAL

GENTIOPICROSIDE AND NH_3.—A solution of gentiopicroside [**2**] (200 mg) in 10 ml EtOH saturated with NH_3 was allowed to stand overnight. The solvent was removed, and 1 N HCl (10 ml) was added. The solution was heated under reflux for 30 min. The acidic solution was alkalinized with NH_3 and extracted with EtOAc. The resinous product (44 mg) was separated by tlc (Si gel G, Et_2O-EtOAc, 4:1). Gentianine [**5**] (17 mg, 18% yield) with $R_f = 0.54$ and gentianidine [**3**] (14 mg; 15%) with $R_f = 0.32$ were isolated.

A solution of gentiopicroside [**2**] (200 mg) in 10 ml EtOH saturated with NH_3 was allowed to stand overnight. The solvent was concentrated to 5 ml, and 10 ml H_2O was added. The alkaloids produced were extracted with EtOAc and purified by tlc. Gentianine [**5**] (11 mg, 11.6%) with $R_f = 0.54$ and gentioflavine [**1**] (24 mg, 22%) with $R_f = 0.10$ were isolated.

GENTIOFLAVINE AND ACID.—A solution of gentioflavine [**1**] (20 mg) in 1 N HCl (10 ml) was refluxed for 30 min. NH_3 was added, the solution extracted with Et_2O, and the extract purified by tlc (Si gel G, Et_2O-EtOAc, 4:1). Gentianidine [**3**] (6 mg, 35%) was isolated.

SWERTIAMARIN AND NH_3.—A solution of swertiamarin acetate (200 mg) in EtOH (10 ml) saturated with NH_3 was allowed to stand overnight. The solvent was concentrated to 5 ml and diluted with H_2O (10 ml). After extraction with EtOAc, the residue (72 mg) was purified by tlc, as described above, to yield gentianine (24 mg, 37%).

GENTIOPICROSIDE AND ETHYLAMINE.—A solution of gentiopicroside (30 mg) in 4 ml 60% $EtNH_2$ in H_2O was allowed to stand overnight. After extraction with EtOAc and purification by

tlc, *N*-ethylgentioflavine [**6**] (4 mg, 22%) with $R_f = 0.21$ was isolated. Eims (70 eV) *m/z* (rel. int.) 211 (4), 210 (4), 194 (9), 176 (100), 148 (45), 147 (53), 120 (65); ^{1}H nmr (250 MHz) ($CDCl_3$) δ 1.20 (3H, d, 7 Hz), 1.35 (3H, t, 9 Hz), 3.03 (2H, t, 6 Hz), 3.5 (2H, m), 4.4 (2H, t, 6 Hz), 4.8 (1H, q, 7 Hz), 7.86 (1H, s), 9.68 (1H, s).

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