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Structure revision of isoline (ruwenine), bisline and isolinecic acid

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Abstract

X-ray crystallography of bisline, and the chemical interconversion of bisline and isoline (ruwenine), revealed that the structures previously assigned to these alkaloids required revision; as did that of isolinecic acid. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Bisline; Isoline; Ruwenine; Ruzorine; Isolinecic acid; Pyrrolizidine alkaloids; *Senecio ruwenzoriensis*; Asteraceae

1. Introduction

The alkaloids ruwenine and ruzorine were isolated by Sapiro (1953) from Kenyan *Senecio ruwenzoriensis* S. Moore (Asteraceae (Compositae)). They were characterized by melting points (mps), and combustion analyses, but were not assigned structures. Subsequently, two pyrrolizidine alkaloids, isoline and bisline, were described as constituents of some populations of South African *S. othonniformis* Foucade, and structures were suggested for them (Coucourakis and Gordon-Gray, 1970). *S. othonniformis* is a synonym of *S. ruwenzoriensis* (Hilliard, 1977), and a reinvestigation of the Kenyan plants led to the conclusion that ruwenine was the same as isoline, while ruzorine was likely to be bisline (Benn and Were, 1992). Evidence is now presented which requires the revision of the structures assigned to these alkaloids.

2. Results and discussion

The structure (**1a**) finally proposed for isoline by Coucourakis et al. (1972) was based on evidence which included saponification or acid-catalyzed hydrolysis to afford products which were identified as retronecine, acetic and isolinecic acid. On the basis of chemical degradation and spectroscopic properties the latter was identified as a 5-ethyl-2,5-dihydroxy-2,3-dimethylhexanedioic acid, the absolute configuration of which was deduced to be (2S, 3R, 5S) (**2a**) by comparison with derivatives of senecic acid (**3**), whose (2R, 3R) absolute stereochemistry had been established by X-ray crystallography (Fridrichson et al., 1960; Koretskaya et al., 1962; Culvenor, 1964). Both acids were degraded to the same (+)-3-methylheptanedione, and formed dilactones which were diastereomers (Coucourakis et al., 1972).

Isoline appeared to be a monoacetate of bisline, although initial attempts to convert bisline to isoline or isoline to bisline were unsuccessful (Coucourakis and Gordon-Gray, 1970). However, the relationship was established when hydrolysis of bisline, isolated from *S. petasitis* D.C. growing in the Canary Islands, yielded isolinecic acid (Gonzalez et al., 1973). Subsequently, we were able to obtain isoline by acetylating bisline (see Section 3). Bisline was thus assigned the structure **4a**.

Although the structures for isoline and bisline appeared to be secure, it was decided to confirm

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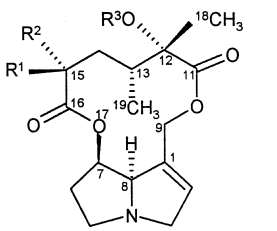
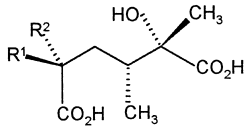
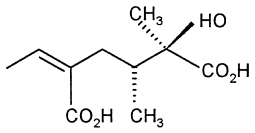


1a R^1 = OH, R^2 = Et, R^3 = Ac
1b R^1 = Et, R^2 = OH, R^3 = Ac
4a R^1 = OH, R^2 = Et, R^3 = H
4b R^1 = Et, R^2 = OH, R^3 = H
6 R^1 = Et, R^2 = R^3 = H



2a R^1 = OH, R^2 = Et
2b R^1 = Et, R^2 = OH



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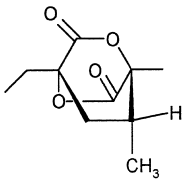

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them by a determination of the crystal structure of bisline, re-isolated from Kenyan *S. ruwenzoriensis*. Crystals suitable for X-ray analysis were grown in acetone–hexanes, and the structure was solved. As a result it was revealed that bisline had the structure **4b**, i.e. the stereochemistry at C-15 was the opposite of that deduced from the chemical evidence. This, in turn, requires the revision of the stereochemistry at C-5 of isolinecic acid, which must be **2b**, and prompted a reexamination of the original derivation of a (5S)-configuration.

Coucourakis et al. (1972) proved that the dilactone obtained from isolinecic acid was **5**, and assumed that the stereochemistry at C-2 and C-5 corresponded to those of isolinecic acid. However, it had been noted (Coucourakis and Gordon-Gray, 1970) that, unlike some other 2,5-dihydroxyhexanedioic acids which possessed the correct stereochemistry for cyclization to dilactones, which they formed spontaneously, isolinecic acid only formed a monolactone when heated with acetic anhydride. Formation of the dilactone occurred when the monolactone was distilled. With the clarity of hindsight, these were significant observations, indicating that the second lactonization was difficult, and occurred with inversion of configuration at C-5, isolinecic acid actually being (2S, 3R, 5R)-5-ethyl-2,5-dihydroxy-2,3-dimethylhexanedioic acid.

In conclusion, the structures of isoline (ruwenine), bisline (with which ruzorine has been tentatively identified), and isolinecic acid have to be revised to **1b**, **4b**, and **2b**, respectively. Bisline can now be visualized as 15-hydroxyyamataimine (**6**), whose structure was established by X-ray crystallography of its hydrobromide salt (Hikichi and Furuya, 1978).

3. Experimental

3.1. General

The mps were uncorr. ^{1}H and ^{13}C-NMR spectra were measured with a Bruker DRX-400 spectrometer of samples dissolved in $CDCl_3$, using solvent resonances at δ_H 7.27 and δ_C 77.0 ppm as internal references. The assignments of ^{1}H and ^{13}C-resonances followed from DEPT, COSY, HMQC, and HMBC correlated-spectra.

3.2. Isolation of the alkaloids

The alkaloids of *S. ruwenzoriensis* were isolated as described previously (Benn and Were, 1992) and a portion (670 mg) was subjected to vacuum short column chromatography over silica gel 60-F254 (Merck) using $CHCl_3$–MeOH–NH_4OH (80:10:1) as eluant, and collecting 10 ml fractions. Individual fractions were evaporated to dryness and then examined by ^{1}H-NMR ($CDCl_3$). Isoline (290 mg) eluted rapidly (F4–7); bisline (40 mg) appeared later (F16–19). After recrystallization from Et_2O-acetone, bisline (**4b**) had mp 168–169°C (Coucourakis and Gordon-Gray, 1970;) mp 169°C), ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 0.81 (3H, *t*, $J = 7.4$ Hz, H-21), 1.18 (3H, *d*, $J = 6.9$ Hz, H-19), 1.23 (3H, *s*, H-18), 1.41 (1H, *dd*, $J = 8.8$ and 14 Hz, H-14B), 1.59 (2H, *q*, $J = 7.4$ Hz, H-20), 1.68 (2H, *m*, H-14A, H-13), 2.13 (1H, *m*, H-6B), 2.29 (1H, *dd*, $J =$ 5.5 and 14 Hz, H-6A), 2.48 (1H, *m*, H-5B), 3.3 (2H, *m*, H-5A and H3B), 4.0 (1H, *dm* 3A), 4.29 (1H, *m*, H-8), 4.43 (1H, *d*, $J = 11.8$ Hz, H-9B), 5.03 (1H, *t*, $J =$ 3.2 Hz, H-7), 5.10 (1H, *d*, $J = 11.8$ Hz, H-9) and 6.15 (1H, *br s*, H-2). δ_C (100 MHz, $CDCl_3$) 7.5 (*q*, C-21), 15.4 (*q*, C-19), 17.5 (*q*, C-18), 33.4 (*t*, C-20), 34.5 (*t*, C-6), 38.9 (*d*, C-13), 41.1 (*t*, C-14), 53.3 (*t*, C-5), 60.1 (*t*, C-3), 63.2 (*t*, C-9), 76.5 (*d*, C-7), 77.4 (*d*, C-8), 78.1 (*s*, C-12), 78.6 (*s*, C-15), 131.6 (*s*, C-1), 135.7 (*d*, C-2), 175.3 (*s*, C-16), 176.1 (*s*, C-11).

3.3. Preparation of isoline

Bisline ((**4b**) 10 mg) was dissolved in a mixture of Ac_2O (0.2 ml) and Py (0.1 ml) and heated at 80°C (both) for 5 h. Water (2 ml) was added to the cold reaction mixture, which was then sonicated briefly. After 30 min the mixture was acidified with 3 M aq. H_2SO_4 (1 ml) and extracted with $CHCl_3$ (3×2 ml). The aqueous phase was basified to pH 9–10 with NH_4OH and extracted with $CHCl_3$ (3×2 ml). The $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to

yield an oil. PTLC (Silica gel 60, F-254 with $CHCl_3$–MeOH–NH_4OH (5:1:0.1) for elution, and I_2 for detection) revealed two major components, R_F 0.6 and 0.3. These were isolated by elution with $CHCl_3$–MeOH–NH_4OH (3:1:0.1), and examined by NMR. The fast moving component (5 mg) proved to be isoline (**1b**) (^{1}H-NMR as shown in Benn and Were (1992); ^{13}C-NMR as shown in Benn and Were (1992) and Drewes et al. (1981)), the slower was recovered as bisline (^{1}H-NMR as in Section 3.2 above).

Under the same conditions, isoline was recovered unchanged with no evidence of conversion to a diacetate.

3.4. Crystal data for bisline

$C_{18}H_{27}NO_6$, MW = 353.41, orthorhombic, space group $P2_12_12$ cell dimensions $a = 11.094(3)$, $b = 18.235(5)$, $C = 9.158(1)$ Å, $V = 1852.6(7))$ Å^3, $Z = 4$, $D_x = 1.267$ Mg m^{-3}, $\mu = 0.784$ mm^{-1}. Intensity data for the colorless crystal ($0.60 \times 0.32 \times 0.30$ mm^3) were collected on an Enraf–Nonius CAD-4 diffractometer using CuK_α radiation ($\lambda = 1.54178$ Å) at 293(2) K. Of 3653 measured reflections, 3368 ($h = 0 \rightarrow 13$, $k = 0 \rightarrow 21$, and $l = -11 \rightarrow 11$) were independent, with 2981 reflections $> 2.0\sigma$(I). Data reduction was carried out with TEXSAN (Molecular Structure); SAPI 91 (Rigaku) was used to solve the structure, and SHELXL 97 to refine it, $R = 4.39\%$. The Flack parameter was 0.0(3). Full details of this structure determination, including atomic coordinates, bond lengths and angles, have been deposited with the Cambridge Crystallographic Data Centre, UK.

Acknowledgements

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