Journal of Natural Products
Vol. 58, No. 10, pp. 1636-1639, October 1995

STRUCTURAL REVISION OF ISOEUDISTOMIN U BY TOTAL SYNTHESIS

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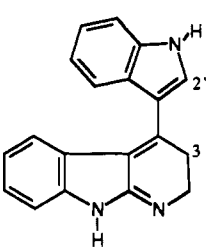
ABSTRACT.—Pictet-Spengler condensation of indole-3-carboxaldehyde and of tryptamine yielded a tetrahydro-β-carboline, which was transformed in one step (DDQ-THF) into a compound whose spectral properties were identical with those of isoeudistomin U, a metabolite from a marine tunicate previously assigned an α-carboline structure. Eudistomin U [**4**] was synthesized from "isoeudistomin U" under the same oxidation conditions but with prolonged reaction time and excess of DDQ.

To the best of our knowledge, the only known natural α-carbolines are grossularines from the marine tunicate *Dendrodoa grossularia* (1,2). Recently, isoeudistomin U [**1**], isolated from the marine ascidian *Lissoclinum fragile*, was added to this family of molecules (3). Unconvinced by the arguments developed in support of structure **1**, we have re-examined these data and now propose structure **2**, that is, 3,4-dihydroeudistomin U, on the basis of a total synthesis.

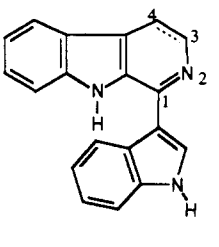
The nmr arguments that were used to propose structure **1** match perfectly a set of formulas of generic structure **3** comprised of a 2,3-disubstituted indole and a 3-substituted indole and containing a six-membered ring with two adjacent methylenes and an imine nitrogen atom. On the basis of primary arguments, isoeudistomin U could be an α, β, γ, or δ-carboline. The rare α-carboline skeleton was originally chosen to explain the observation of an nOe between H-2′ of the 3′-substituted indole nucleus and H-3 of the α-carboline. The magnitude of the effect (12% at 400 MHz), however, casts suspicion upon the interpretation of the experiment and suggests an experimental artifact and/or concomitant saturation of the end tail of the H_2O peak and relay through exchangeable protons. The chemical shift of C-9a (158.6 ppm) also initially supported the α-carboline nature of the molecule but the original assignment was recently revised and this atom now appears at 148.6 ppm (personal communication from M. Guyot, CNRS MNHN, Paris).

Another argument in favor of the dihydro-α-carboline structure was the observation of long-range C-H coupling between C-9a and H-2 and C-4a and H-3. Reversal of structures and permutation of assignments allows the same deduction to be made on β and δ-carboline skeletons, but not on a γ-carboline skeleton, as indicated in Scheme 1.

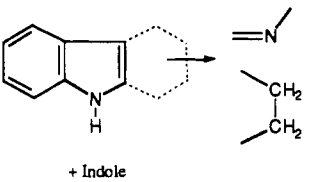
The simultaneous isolation of β-



1 (Previous structure of isoeudistomin U)



2 Isoeudistomin U (this work)



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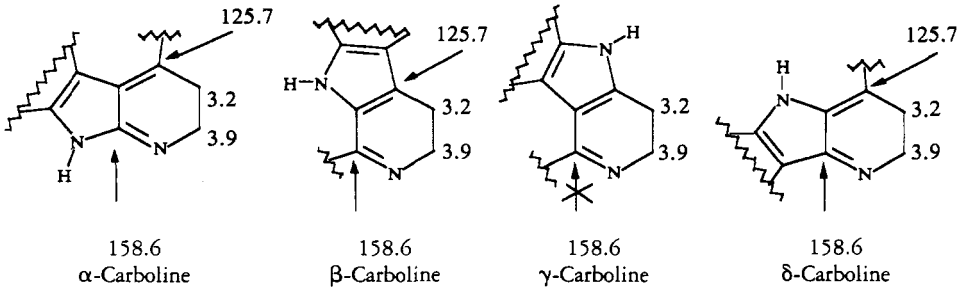
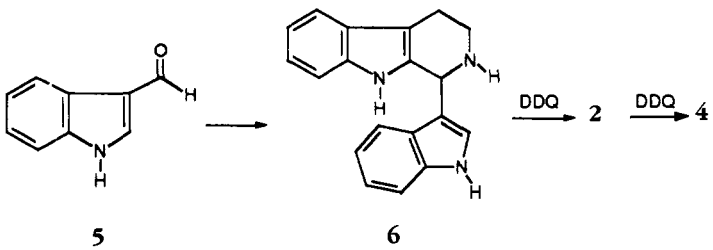


SCHEME 1. Explanation of long-range couplings by reversal of structures and permutation of assignments.

carboline **4** (eudistomin U) from the same organism and the observation in the ^{13}C-nmr spectrum of a methylene at δ 20.3 ppm suggested that isoeudistomin U was a dihydro-β-carboline. This hypothesis was strengthened by comparison of uv and ms data for **4** and for isoeudistomin U. The uv spectra of the two compounds showed important similarities, which is unexpected given the different length and nature of the chromophore in the proposed structure. This feature could be explained by contamination of one compound by the other, an obvious feature of the mass spectrum of **1**, which shows a $[M-2H]^+$ ion. Synthesis of the alternative dihydro-β-carboline structure [**2**] appeared a realistic way to settle the issue, and was therefore undertaken to clarify the situation.

A retrosynthetic analysis identified tryptamine and indole-3-carboxaldehyde [**5**] as precursors for **4** (Scheme 2). The carbonyl function in **5** does not behave as a genuine aldehyde but rather as a vinylogous amide. 3-Formylindoles have been used on several occasions by Magnus as imine-like intermediates after protection of the nitrogen atom as a benzenesulfonamide (4). In this particular case, however, protection was found unnecessary and stirring tryptamine and **5** in toluene under reflux gave, upon evaporation of the solvent, an imine which was not purified but rather directly cyclized into tetrahydro-β-carboline **6** under acidic conditions (TFA, $CHCl_3$, 93% isolated yield). Oxidation of **6** to the corresponding β-carboline was achieved uneventfully under "dry Yonemitsu" (4) conditions (THF, DDQ), and the use of 1.12 equivalents of DDQ gave a non-optimized yield of 70% of **2**. Further oxidation to **4** only occurred under more forcing conditions (prolonged reaction time and 4 equivalents of DDQ, 78% yield).

Scarcity of the natural product did not allow a direct comparison of samples and identification had to be based on spectral evidence. The eims data of **2** (synthetic) showed intense molecular ion and $[M-1]^+$ peaks and less intense peaks corresponding to $[M-2]^+$ and $[M-3]^+$ (25% of molecular ion); the only meaningful fragmentation corresponded to the



5 **6**

SCHEME 2. Synthesis of "isoeudistomin U" [**2**] and of eudistomin U [**4**].

loss of a fragment of 28 mass units (C_2H_4). The uv spectrum of the synthetic material matched the one of the natural product (λ max at 250, 275, 281, 350, 395 nm); the absorption at 450 nm, not present in the synthetic product, might be due to contamination by **4**.

The ^{1}H-nmr spectrum of synthetic **2** showed signals with the same multiplicities as and chemical shifts similar to the natural product. Worthy of note are the signals for the methylenes at δ 3.35 and 4.1 ppm, which would have been expected to undergo significantly different chemical shifts in alternative structures.

Comparison of the ^{13}C-nmr spectra was more difficult to obtain. The original measurements on the synthetic compound were obtained in a mixture of $CDCl_3$ and CD_3OD (0.25 ml each) in the presence of one drop of TFA for solubility, and chemical shifts, and assignments listed in Table 1 are based on ^{1}H-^{1}H COSY, HMQC, and HMBC experiments. Deviations were observed from the published data and a new set of assignments (Table 1) was obtained in CD_2Cl_2 as in Badre *et al.* (3) with one drop of TFA added. Despite superimposition of signals, which made definitive assignments more difficult to make, we believe that the synthetic and natural products are identical and that "isoeudistomin U" is in fact dihydroeudistomin U. The two natural products from *Lissoclinum fragile* thus have the same biosynthesis. Synthetic **2** was inactive in a tubulin bioassay (6).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded at 300 and 75 MHz, respectively, on a Bruker AC 300 spectrometer equipped for inverse detection; ms were measured on a JEOL DNM300 instrument.

Tetrahydro-β-carboline **6**.—A mixture of indole-3-carboxaldehyde (1.99 g, 13.72 mmol) and tryptamine (2 g, 12.41 mmol) in 50 ml of toluene was stirred for 1 h under reflux. The solvent was evaporated and the residue dissolved into 20 ml of $CHCl_3$. The mixture was treated with a mixture of TFA (10 ml) and $CHCl_3$ (20 ml) and stirred at room temperature for 24 h, neutralized by addition of aqueous Na_2CO_3 and extracted with $CHCl_3$. The organic layers were washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was

TABLE 1. ^{13}C-Nmr Assignments for Natural and Synthetic Isoeudistomin U [**2**] [values from Badre *et al.* (3) are assigned according to revised structure and appropriate numbering].

Carbon	Badre *et al.* (3)	Present work		
	(CD_2Cl_2-TFA)	($CDCl_3$-CD_3OD-TFA)	(CD_3OD-TFA)	(CD_2Cl_2-TFA)
1	158.6	157.3	158.2	157.0
3	42.3	41.9	42.8	42.2
4	20.4	20.5	20.6	20.2
4a	124.9	125.6	125.7	125.3
4b	125.7	125.5	125.8	125.3
5	121.7	121.6	122.2	121.7
6	122.7	122.3	122.8	122.7
7	129.7	128.9	129.1	129.6
8	113.7	113.6[a]	114.2	113.8
8a	142.9	142.0	142.6	141.7
9a		113.6	114.1	113.7
1′	109.4	106.5	107.6	106.2
2′	138.0	137.4	137.7	137.8
3′a	141.1	138.2	139.1	139.5
4′	114.4	113.8[a]	114.3	114.2
5′	124.9	123.8	124.1	124.2
6′	124.2	125.5	125.6	125.3
7′	119.1	120.2	120.9	119.2
7′a		124.7	126.7	124.2

[a]These values may be interchanged.

purified by cc on Si gel eluted with $CHCl_3$-MeOH (2:3) to give compound **6** as a pale yellow solid (3.33 g, 93%); ^{1}H nmr ($CDCl_3$) δ 8.42 (1H, br s, NH), 7.85 (1H, br s, NH), 6.75 (1H, s, H-2′), 3.33 (1H, m, H-3), 3.10 (1H, m, H-3), 2.84 (2H, m, H_2-4), 1.80 (1H, br s, NH); ^{13}C nmr ($CDCl_3$) δ 136.4, 135.6, 135.2, 127.5, 126 (5 C), 123.8, 122.3, 121.4, 119.9, 119.2, 119.0, 118.1 (7 CH), 115.9 (C), 111.4, 110.9 (2 CH), 109.1 (C), 50.2 (CH), 43.1, 22.5 (2×CH_2); eims *m/z* $[M]^+$ 287 (40), 286 (30), 258 (25), 257 (25), 170 (70), 169 (100), 117 (95).

Isoeudistomin U [**2**].—To a solution of **6** (200 mg, 0.7 mmol) in 4 ml of THF, was added dropwise a solution of DDQ (177 mg, 0.78 mmol) in 2 ml of THF. The mixture was stirred at room temperature for 1 h. Filtration of the mixture and evaporation of the solvent followed by cc on Si gel with $CHCl_3$-MeOH (2:3) as eluent, afforded **2** as a pale yellow solid (140 mg, 70%); uv λ max (MeOH) 250, 275, 281, 350 (sh), 395 nm, (MeOH+CF_3CO_2H) 254, 268, 281, 356 (sh), 396 nm; ^{1}H nmr (CD_3OD, CF_3CO_2H) δ 8.28 (1H, s, H-2′), 7.96 (1H, m, H-7′), 7.72 (1H, br d, J=8.2 Hz, H-5), 7.62 (1H, m, H-4′), 7.59 (1H, d, J=8.4 Hz, H-8), 7.45 (1H, br t, J=8.3 Hz, H-6), 7.38 (2H, m, H-5′, H-6′), 7.23 (1H, br t, J=8 Hz, H-7), 4.10 (2H, t, J=7.7 Hz, H_2-3), 3.35 (2H, t, J=7.7 Hz, H_2-4); ^{13}C-nmr data, see Table 1; eims *m/z* $[M]^+$ 285 (100), 284 (99), 283 (20), 282 (25), 257 (50), 256 (45).

Eudistomin U [**4**].—DDQ (15 mg, 4 equivalents) was added to a solution of synthetic **2** (4 mg) in 0.5 ml of THF. The mixture was stirred overnight at room temperature and treated as above. Eudistomin U [**4**] was obtained pure by filtration on Si gel (3.6 mg, 78%). Spectral properties matched those reported previously (3).

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Received 2 May 1995