

ISOLATION OF TWO NEW NITROGENOUS METABOLITES FROM THE CULTURED CELLS OF ASPIDOSPERMA QUEBRACHO-BLANCO

Norio Aimi,*^a Naoki Uchida,^a Naoko Oya,^a Shin-ichiro Sakai,^a Luis A. Mendonza,^b Peter Obitz,^c and Joachim Stöckigt*^c

a) Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inageku, Chiba 263, Japan

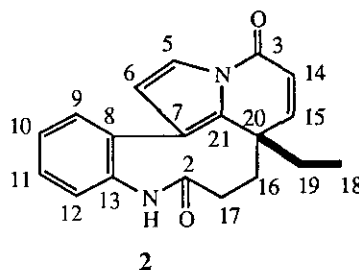
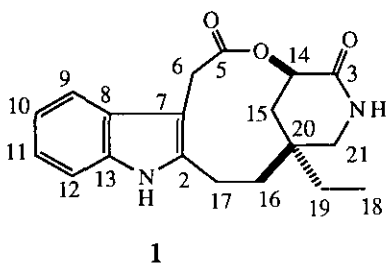
b) I. N. T. A., Centro de Investigaciones de Recursos Naturales, Casturales, Argentina

c) Institute of Pharmacy, Johannes Gutenberg-Universität Mainz, Staudinger Weg, 55099 Mainz, Germany

Abstract Two novel nitrogen containing metabolites were isolated from cultured cells of *Aspidosperma quebracho-blanco*. Their structures were elucidated as 11-hydroxytubotaivine and 1,6-propano-3-ethylideno-1,4-piperazine-2,5-dione.

Aspidosperma quebracho-blanco (Apocynaceae) attracts the continuing interest of natural product chemists because of its importance as the folk medicine employed in South American countries and its production of a rich variety of monoterpenoid indole alkaloids.¹ The importance of this plant species stimulated us to develop tissue and cell suspension cultures. We succeeded in establishing a cell strain which shows steady growth as a fine cell suspension in liquid media.²

In our previous paper² we described the isolation and structure determination of two new monoterpenoid indole alkaloids from these cultured cells. Aspidochibine (**1**) was found to be a new type of *Aspidosperma* alkaloid which is considered to be biosynthesized from a member of quebrachamine class of alkaloids through oxidation at C-5, hydrolytic cleavage of the resulting amide linkage between C-5 and N₆, and macrocyclic lactone formation between the C-5 carboxylic acid and the hydroxyl group on C-14. The stereochemistry and the conformation of this molecule were also studied. Another new

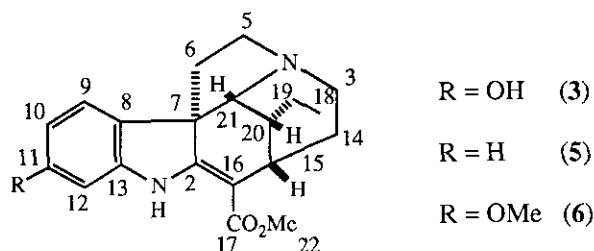


compound was 14,15-dehydro-3-oxo-rhazinilam (**2**), an alkaloid with a highly advanced oxidation level and belonging to a very small group of alkaloids (rhazinilams).

In our further studies on the metabolites of the cultured cells of *Aspidosperma quebracho-blanco* we have now isolated two new nitrogen containing metabolites and determined their structures

The compound AQC-20 (**3**) (2.2 mg) was isolated from a basic fraction of the methanol extract obtained from the lyophilized cultured cells of the named plant. The production yield of **3** was almost comparative to those of **1** and **2** in the cultured cells. The high resolution ms revealed the molecular formula of **3** to be $C_{20}H_{24}N_2O_3$. The uv spectrum showed the absorption maxima at 255 and 328 nm which clearly indicated the presence of the β -aminoacrylic ester chromophore. The fragmentation pattern of EI-ms of **3** strongly suggested that the molecule is a member of tubotaiwine group of compounds³ having one additional oxygen to the molecule of tubotaiwine (**5**).

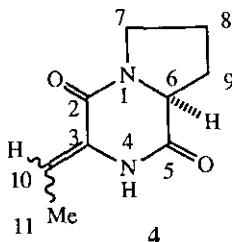
The comparison of the ^{13}C -nmr spectra of AQC-20 with that of tubotaiwine⁴ (**5**) revealed their close similarity. The signals of the carbons of the non-aromatic part of **3** were almost superimposable on those of tubotaiwine. This observation clearly indicated that the additional oxygen is located on the aromatic ring as a phenolic hydroxyl group. Next the location of the hydroxyl group was clarified. In the 1H -nmr spectrum an aromatic proton was observed at δ 6.46 as a singlet. The corresponding carbon showed the chemical shift of δ 95.9 ppm, a shift position which indicates a strongly shielded aromatic ring carbon. The location of the oxygen at C-11 is in excellent agreement with such a shielding effect to the neighboring position. Moreover, close similarity of the aromatic carbon shifts of **3** with the described carbon shifts for 11-methoxytubotaiwine⁵ (**6**) strongly supported this structural assignment.



Tubotaiwine has been found to be a constituent of many Apocynaceae plants and has also been isolated from some cell suspension cultures. It was found in suspension cultures of *Stemmadenia tomentosa* Grenman var. *plmeri*,⁶ *Tabernaemontana divaricata* (L.) R. Br. ex Roem. et Schult., and *T. iboga* Baill.⁷ The stereochemistry of the molecule, in particular the configuration of the chiral center at C-20 was unsettled for many years. From a broad survey of described isolations of tubotaiwine and by means of NOE, ^{13}C -H coupling constants and protonation shifts it was concluded that all natural tubotaiwines possess *S* configuration at C-20.⁴ Close similarity of the shift values and coupling constants of the carbons and protons around C-20 together with the big dextrorotatory $[\alpha]_D$ value strongly indicated that AQC-20 (**3**) belongs to the same stereochemical group as the known natural tubotaiwines. All nmr-data were in complete agreement with the structure (**3**) as illustrated

The $CHCl_3$ eluant from SiO_2 column purification of the basic fraction and further purification by mpc (medium pressure liquid chromatography) gave the second cellular metabolite, AQC-14 (**4**). High resolution ms measurement indicated that the molecular formula was $C_9H_{12}N_2O_2$. The 1H -nmr disclosed the presence of an ethylidene group by the doublet for the methyl

group at δ 1.75 ($J = 7.4$ Hz) and a quartet for the vinyl H at δ 6.13 ($J = 7.4$ Hz). Furthermore a series of seven protons on four sequential carbons was observed. The last proton, which was found at δ 7.45 as a broad singlet, was exchangeable with D_2O . This proton was reasonably ascribable to an amide NH. These observations are consistent with the dioxopiperazine structure (4) as shown.



The uv spectrum possessing an absorption maximum at 224 nm also strongly supports the depicted structure which carries a conjugated amide chromophore ⁸. All the nmr-data, including the ¹³C-values, clearly support the deduced structure. It is most likely that this molecule has been formed through condensation of L-proline and L-threonine followed by elimination of one molecule of water. In accord to this view the absolute configuration at C-6 is safely postulated as shown. The configuration of the double bond remains to be determined. At this moment, however, we can not exclude the possibility that 4 is a secondary product resulting from condensation of the corresponding amino acids under the isolation conditions.

EXPERIMENTAL

The nmr spectra were run on a JEOL JNMA500 or a JNM-GSX400 instrument in $CDCl_3$ with tetramethylsilane as an internal standard. The mass spectrometer used for the measurements of EI- and FAB-ms was a JEOL JMS HX-110. For measurement of FAB-ms *m*-nitrobenzyl alcohol was used as the matrix. Adsorbent for open column chromatography was silica gel G, Merck. For flash column chromatography silica gel 60, Merck, 230-400 mesh, was used. A prepacked column Si-5, Kusano Kagaku Kikai Co. was used for medium pressure liquid chromatography. Plant cell suspension cultures were performed in the LS and 4X- medium as previously described ².

Isolation of AQC-20 (3) and AQC-14 (4)

Extraction of the cultured cells (98.2 g) with hot methanol (2 l, 1.5 l, 1.5 l) for each 24 h gave the crude extract (25.4 g), which was then dissolved in 1N-HCl. After being washed with AcOEt the aqueous layer was basified with conc NH_4OH . Extraction with $CHCl_3$ gave a crude residue (129 mg). Flash column chromatography separation of the residue gave a syrup on elution with $CHCl_3$. Repeated separation of the syrupy residue gave AQC-14 (4) as a homogeneous material (3 mg). Fractions eluted from the above open column with 20-40 % MeOH in $CHCl_3$ were combined. Separation of the residue with repeated medium pressure liquid chromatography afforded AQC-20 (3) (2.2 mg) as a homogeneous syrup.

AQC-20 (11-Hydroxytubotaiwine) (3): An amorphous powder, $C_{20}H_{24}N_2O_3$. High resolution FAB-ms, Found m/z ; 341.1872; Calcd for $C_{20}H_{25}N_2O_3$ ($M + H$)⁺; m/z 341.1865. Uv λ_{max} (EtOH), 255 (log ϵ 3.78) and 328 (log ϵ 3.87) nm. λ_{max} (EtOH + NaOH), 285 nm. $[\alpha]_D^{+207}$ ($c = 0.04$, EtOH). EI-ms m/z (%): 340 (M^+ , 11), 283 (5), 245 (11), 196 (11), 183 (8), 167 (5), 154 (6), 115 (15), 95 (33), 83 (39), 71 (100). ¹H-Nmr (400MHz, $CDCl_3$) δ , 2.94 (ddd, $J = 12.1, 12.1, 5.0$

H_z, H-3), 3.60 (m, H-3), 3.16 (ddd, J = 11.5, 7.3, 2.4 Hz, H-5), 3.60 (m, H-5), 2.19 (m, H-6), 2.76 (ddd, J = 14.1, 11.5, 7.5 Hz, H-6), 7.02 (d, J = 8.4 Hz, H-9), 6.46 (d, J = 8.6 Hz, H-10), 6.46 (s, H-12), 1.97 (m, H-14), 2.15 (m, H-14), 3.23 (br s, H-15), 0.69 (3H, dd, J = 7.2, 7.2 Hz, 18-Me), 0.85 (2H, m, H-19), 2.19 (m, H-20), 4.43 (br s, H-21), 3.80 (3H, s, COOMe), 8.75 (s, NH), 12.4 (br s, OH). ¹³C-Nmr (100 MHz, CDCl₃) δ : 160.0 (C-2), 43.9 (C-3), 53.7 (C-5), 45.2 (C-6), 54.4 (C-7), 128.7 (C-8), 120.2 (C-9), 107.5 (C-10), 156.2 (C-11), 98.4 (C-12), 144.9 (C-13), 28.4 (C-14), 30.8 (C-15), 95.9 (C-16), 170.9 (C-17), 11.6 (C-18), 23.8 (C-19), 41.2 (C-20), 65.3 (C-21), and 51.1 (C-22).

AQC-14 (1,6-Propano-3-ethylideno-1,4-piperazine-2,5-dione) (4) · An amorphous powder. C₉H₁₂N₂O₂. High resolution EI-ms; Found 180.090, Calcd for C₉H₁₂N₂O₂ (M⁺); 180.090. Uv $\lambda_{\max}$ (EtOH); 224 nm. ¹H-Nmr (500 MHz, CDCl₃) δ : 1.75 (3H, d, J = 7.4 Hz, 11-Me), 6.13 (q, J = 7.4 Hz, 10-H), 4.19 (dd, J = 10.2, 6.6 Hz, 6-H), 3.58 (ddd, J = 12.4, 9.3, 3.0 Hz, 7a-H), 3.7 (ddd, J = 12.5, 8.3, 8.3 Hz, 7b-H), 1.9 - 2.1 (3H, m, 8a, 8b, 9b-H), 2.44 (dd, J = 10.4, 6.4 Hz, 9a-H), 7.45 (br s, NH). ¹³C-Nmr (100MHz, CDCl₃) δ : 158.0 (C-2), 128.8 (C-3), 166.1 (C-5), 59.0 (C-6), 45.3 (C-7), 21.8 (C-8), 28.9 (C-9), 113.3 (C-10), 11.0 (C-11).

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