Communications to the editor

ARGVALIN, A NEW MICROBIAL METABOLITE: ISOLATION AND STRUCTURE

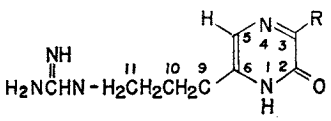
Sir:

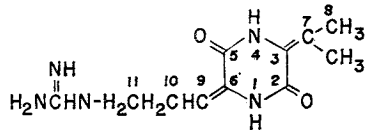
As reported in previous papers[1,2,3,4,5], chemical screening of microbial culture filtrates gives new compounds of structural interest. In this paper, we report the isolation and structural studies of argvalin (**1**) which was discovered by searching for compounds with positive WOOD[6] and diacetyl color-reactions.

The culture filtrate (10 liters, pH 6.7) of a strain, KG 62-AGl which was closely related to *Streptomyces filipinensis**, was extracted with *n*-butanol at pH 8 and the extract was purified by successive application of column chromatography on acidic alumina (with methanol), cellulose [with *n*-butanol-ethanol-water (6:1:2)] and Dowex 1×2 (OH form) (with water) followed by neutralization with hydrochloric acid. Recrystallization from isopropyl alcohol and ethyl acetate gave colorless needles of argvalin monohydrochloride (200 mg), mp 175~176°C, $[\alpha]_D^{24}$ 0° (*c* 2.0, water); pKa (60% aq. DMF): <4, 10.0 and >13; Anal. Found: C 47.98, H 7.32, N 25.01, Cl 12.73%: Calcd. for $C_{11}H_{19}N_5O \cdot HCl$: C 48.26, H 7.36, N 25.58, Cl 12.95%; TLC with cellulose: Rf 0.8 [*n*-BuOH - AcOH - H_2O (12 : 3 : 5)]; Rf 0.6 [*n*-BuOH - EtOH - $CHCl_3$ - 17% NH_4OH (4 : 4 : 2 : 3)]; Rf 0.55 [*n*-BuOH - EtOH - H_2O (6 : 1 : 2), arglecin[3] (2) gave Rf 0.60].

Argvalin is a basic substance and positive to WOOD, diacetyl and SAKAGUCHI, but negative to ninhydrin and EHRLICH reagents. It may also be detected by ultraviolet light (blue fluorescence). On high voltage paper electrophoresis (3,500 V/42 cm, 90 mA/20 cm, 15 min.; Toyo Roshi paper No. 51) with a buffer solution of HCOOH - AcOH - H_2O (1 : 3 : 36), it showed the same mobility (8.5 cm toward the cathode) as that of arglecin (**2**). IR (KBr): 3350, 3200 (NH); 2950 (CH); 1680, 1620 cm^{-1} (C=O, C=C and guanidinium); UV, $\lambda_{max}^{H_2O}(\varepsilon)$: 321 (9,600), 226 mμ (8,300); $\lambda_{max}^{0.1N\ HCl}(\varepsilon)$: 333 (9,600), 226 mμ (9,100); Mass (*m/e*), 237 (M^+), 209, 178, 165, 163, 150, 149, 137, 135, 126, 121, 86, 73; NMR [D_2O; δ value (ppm)]: 1.20 [6H d, *J* 7 Hz, CH-(CH_3)$_2$], ~2.0 (2H m, CH_2-10), 2.71 (2H t, *J* 7 Hz, CH_2-9), 3.29 (2H t, *J* ~6.5 Hz, CH_2-11), ~3.4 (1H m, H-7), 7.3 (1H s, H-5).

By the NMR spectrum, the presence of a trimethylene group -CH_2(9) - CH_2(10) - CH_2(11) - and an isopropyl group -CH(7) - $(CH_3)_2$ was indicated. A long-range coupling (J<1 Hz) as well as nuclear OVERHAUSER effect between H-5 and H-9 were also observed as seen in arglecin[3b]: irradiation at δ 2.7 (H-9) caused change of the singlet at δ 7.3 (H-5) to a sharpened singlet with an increased absorption of 17%. When argvalin was refluxed in 1 N deuterochloric acid for 4 hours, the H-7 proton was deuterated as found with arglecin (**2**). These NMR and other spectral data suggested





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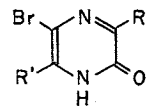
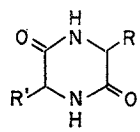
Argvalin (1): R = -CH(CH$_3$)$_2$

Arglecin (2): R = -CH$_2$CH(CH$_3$)$_2$





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(R = -CH(CH$_3$)$_2$ R' = -CH$_2$CH$_2$CH$_2$-NHC(=NH)NH$_2$)

* This taxonomy was performed by Dr. MASA HAMADA of Institute of Microbial Chemistry.

that argvalin has 2(1H)-pyrazinone structure.

In order to confirm structure **1**, argvalin was converted into the corresponding dioxopiperazine in a similar way as described for arglecin[3b]. A solution of argvalin in 80% aqueous acetic acid was treated with bromine at room temperature for 1 hour and the resulting solution was evaporated to dryness. The residual solid was dissolved in boiling water and allowed to stand at room temperature to give a precipitate of the monohydrobromide of 6-(3-guanidinopropylidene)-3-isopropylidene-2, 5-dioxopiperazine (**3**) in 21% yield, which was recrystallized from 95% aqueous acetic acid, mp 286°C (dec.); Mass (M^+), 251; UV, $\lambda_{max}^{H_2O}$ (ε) 289 (22,800), 243 (sh., 7,300), 235 mμ (sh., 7,000); NMR (DMSO-d_6): δ 1.85 and 2.20 [each 3H s, $=C(CH_3)_2$], ~2.5 (2H m, CH_2-10), 3.2 (2H broad t, J~7 Hz, CH_2-11), 5.7 (1H t, J~7 Hz, CH-9), 7~7.5 [4H m, -NHC(=NH)NH_2], 9.58 and 9.95 (each 1H broad s, NH).

The filtrate was evaporated and the residue chromatographed on cellulose with *n*-BuOH-EtOH-H_2O (6:1:2) to give the monohydrobromide of monobromo derivative (**4**) in 34% yield. Product 4 was recrystallized from acetone, mp 169°C (dec.); Mass (M^+), 317 and 315; UV, $\lambda_{max}^{H_2O}$ (ε) 334 (7,600), 234 mμ (8,700); NMR (D_2O): δ 1.20 [6H d, J 7 Hz, CH-$(CH_3)_2$], ~2.0 (2H m, CH_2-10), 2.83 (2H t, J~7 Hz, CH_2-9), 3.34 (2H t, J~7 Hz, CH_2-11), ~3.4 (1H m, CH-7).

Compound **3** was hydrogenated with platinum oxide in 50% aq. acetic acid and the product was chromatographed on a Dowex 1×2 (OH form) with water. The eluate was neutralized with 0.1 N hydrochloric acid and evaporated to give a solid of the corresponding dioxopiperazine (**5**) which was recrystallized from ethanol-isopropyl ether, 81% yield, mp ~152°C (dec.); $[\alpha]_D^{20}$ 0° (*c* 2, water). Product **5** was also obtained from **4** by treatment with zinc dust in 80% aq. acetic acid for 1 hour in a yield of 62%. When product **5** was hydrolyzed with 48% hydrobromic acid overnight in a sealed tube at 110°C, valine and arginine were obtained, indicating that **5** is a 2, 5-dioxopiperazine composed of valine and arginine. To confirm the structure of **5**, 3S : 6S-6-(3-guanidinopropyl)-3-isopropyl-2, 5-dioxopiperazine [**5'**; mp ~152°C (dec.), $[\alpha]_D^{22}$ −11.3° (*c* 1.0, water)] was synthesized from nitro-L-arginine ethyl ester monohydrochloride[5] and *t*-butyloxycarbonyl-L-valine *N*-hydroxysuccinimide ester[7] in four steps by methods described in a previous paper on arglecin[3b]. The intermediary 3S : 6S-3-isopropyl-6-(3-nitroguanidinopropyl)-2, 5-dioxopiperazine had mp ~212°C (dec.), $[\alpha]_D^{16}$-50° (*c* 1.0, acetic acid). The IR and NMR (DMF-d_7) data of **5** and **5'** were identical.

From the above results, the structure of argvalin was determined to be 6-(3-guanidinopropyl)-3-isopropyl-2(1H)-pyrazinone (**1**).

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