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THE STRUCTURE OF ARGLECIN, A NEW METABOLITE OF *STREPTOMYCES*

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As the results of our screening of culture filtrates of microorganisms for chemical color reactions to find new metabolites, dienomycins (1) and sphydrofuran (2) have already been found as metabolites of *Streptomyces*.

We now report the structural elucidation of a new metabolite, arglecin (I) produced by a wide variety of *Streptomyces* and found by a screening with Wood reagent (3).

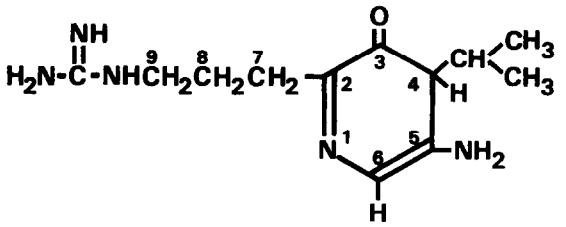
The culture filtrates of *Streptomyces* were extracted with butanol and the resulting mass was purified by column chromatography with alumina and ion-exchange resin to give crude arglecin. Recrystallization from ethanol - ethyl acetate gave colorless needles of arglecin (I)·dihydrochloride: mp 176∿177°C; $[\alpha]_D^{20}$ 0° (*c* 2.5, H_2O); Found: C, 44.64; H, 7.32; N, 21.42; O, 5.12; Cl, 21.50%; M.W., 251 (mass spectrum, M^+); Calcd. for $C_{12}H_{21}N_5O$ (M.W., 251.33)·2HCl: C, 44.45; H, 7.15; N, 21.60; O, 4.93; Cl, 21.87%; IR (KBr): 3360, 3200 (NH); 2980∿2660 (CH), 2040 (immonium); 1695, ∿1655, 1635, 1590cm^{-1} (C=O, C=C and guanidinium); UV: $\lambda_{max}^{H_2O}$ (ε) 322 (9,600), 226mμ (8,000); $\lambda_{max}^{0.1N\ NaOH}$ (ε) 321 (9,100), 232mμ (8,200); $\lambda_{max}^{0.1N\ HCl}$ (ε) 338 (9,400), 226mμ (8,700); TLC: R_f 0.6 [silica gel, n-BuOH-EtOH-H_2O (5 : 1 : 2)]; R_f 0.8 [cellulose, n-BuOH-AcOH-H_2O (12 : 3 : 5)]; pka 9.1 and >11 (H_2O).

The NMR spectrum (Table 1) of arglecin dihydrochloride (in D_2O) was analyzed with aid of double resonance experiments and the presence of the following groups were shown: one isopropyl (δ 1.01, 6H d; δ 2.18, 1H m), one trimethylene (δ 2.06, 2.87 and 3.36, each 2H), one methine (δ 2.84, 1H d) and one olefinic (δ 7.50, 1H s) groups and six active hydrogens exchangeable for deuteriums. When the solution of the dihydrochloride of I in deuterium oxide was allowed to stand at 5°C for a week or the solution of I in 1N deuterochloric acid in deuterium oxide was refluxed for 1hr, a doublet of the methine proton at δ 2.84 (H-4) disappeared without disturbance

Table 1. NMR data (δ values)

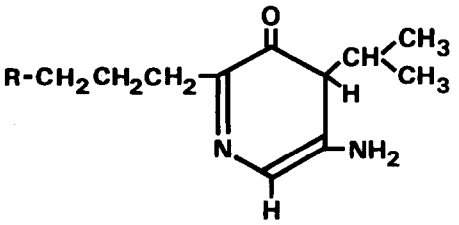
Compound	I	II	III	IV	V
Solvent	D_2O	D_2O	$CDCl_3$	D_2O	D_2O
H - 2				4.04 t (∿6)	
H - 4	2.84 d (7)*	2.85 d (7.5)	2.67 d (7.5)	∿1.8	∿1.8
H - 5				3.87 m	
H - 6	7.50 s	7.49 s	7.28 s	3.30 q (8, 13)	3.30 s
H - 6'				3.59 q (4.5, 13)	
CH_2 - 7	2.87 t (7.5)	2.89 t (7.5)	2.58 t (7)	∿1.7	∿1.7
CH_2 - 8	2.06 m (∿7)	∿2.1	∿2.0	∿1.7	∿1.7
CH_2 - 9	3.36 t (6.5)	3.19 t (∿7)	3.37 q (∿6)	3.21 t (6)	3.21 t (6)
$\underline{CH_3}>CH-$	1.01 d (7)	0.99 d (7)	0.96 d (7)	0.98 d (6), d (6)	0.98 d (6), d (6)
$CH_3>\underline{CH}-$	2.18 m	∿2.2	∿2.0	∿1.8	∿1.8
$\underline{CH_3}CONH-$			2.00 s	1.99 s	1.99 s
-NH-			∿6.5		

* Coupling constants (Hz) are shown in parentheses.



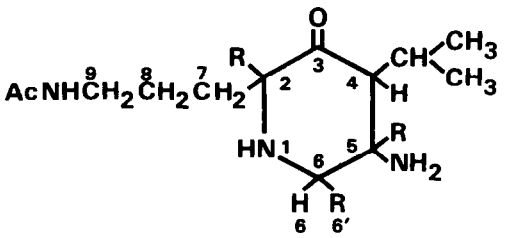
ARGLECIN

(I)



II : R = $-NH_2$

III : R = $-NHCOCH_3$



IV : R = −H

V : R = −D

of any other signals. However, when the above solutions were refluxed in 1N hydrochloric acid in water for 1hr, the doublet was recovered. This phenomenon suggested the presence of a carbonyl group situated adjacent to the methine, to which an isopropyl group is attached. On the other hand, the diacetyl and Sakaguchi - positive characters of I indicate, together with its pka values, the presence of a guanidino and an aromatic amino groups.

When arglecin was treated with barium hydroxide followed by neutralization with hydrochloric acid, a diacetyl and Sakaguchi - negative derivative (II) was obtained as a dihydrochloride·monohydrate: $C_{11}H_{19}N_3O$ (209.29)·2HCl·H_2O; M.W., 209 (M^+); mp 232∿234°C (recrystallized from EtOH - EtOAc); pka 8.9 and 10.6. This compound (II), from the above results and its ninhydrin - active character, must possess an aliphatic and an aromatic amino groups. There exists no significant change between the NMR spectra of I and II except for the chemical shift of a methylene triplet at δ 3.19 (H-9), which shifts to the upper-field by 17Hz (Table 1).

Acetylation of II with acetic anhydride and pyridine gave a mono-N-acetylated compound (III): $C_{13}H_{21}N_3O_2$ (251.32); M.W., 251 (M^+); mp 180∿181°C (from EtOAc - MeOH); pka 9.4, which showed that the aromatic amino group still remained unacetylated.

The NMR spectrum of III (in $CDCl_3$) showed a two-proton quartet (J ∿6Hz) at δ 3.37 (H-9), which collapsed to a triplet upon deuteration with D_2O. This indicates that the methylene (δ 3.37) bears an acetamido group and therefore a guanidino group in I. From the above-mentioned results, the presence of the following groups as $H_2NC(=NH)NHCH_2CH_2CH_2\overset{|}{C}-$, $-\overset{|}{C}OCH(CH_3)_2$, -CH=, $=\overset{|}{C}NH_2$ and probably =N- were concluded. Since the chemical formula of I ($C_{12}H_{21}N_5O$) has five unsaturation number, the presence of the above groups indicates that I has a ring system having two double bonds in it, to which a guanidinopropyl, an isopropyl, an amino and an oxo groups are attached. The next problem is, therefore, to decide the positions of the above-described groups on the ring, and the species of the ring.

Hydrogenation of III with platinum oxide readily gave a tetrahydro derivative (IV) as a monohydrochloride: $C_{13}H_{25}N_3O_2$ (255.35)·HCl; M.W., 255 (M^+); mp 201∿202°C (from EtOH - EtOAc); IR (KBr): 1700 (C=O), 1660 (amide I), 1550cm^{-1} (amide II), which retains a carbonyl group.

On the other hand, treatment of III with deuterium and platinum oxide gave a trideuterated derivative (V) as a monohydrochloride: $C_{13}H_{22}D_3N_3O_2$ (258.38)·HCl; M.W., 258 (M^+); mp 200∿201°C (from EtOH - EtOAc); IR (KBr): 1700 (C=O), 1660 (amide I), 1550cm^{-1} (amide II). On TLC [silica gel, EtOAc-MeOH (2 : 1)], IV and V showed identical mobilities (R_f 0.3).

In IV, three protons, which were not discerned in V and therefore considered to be produced on the hydrogenation of the double bonds of III, appeared at δ 4.04 (triplet, H-2), 3.87 (multiplet, H-5) and 3.59 (quartet, H-6'). On the other hand, another quartet (δ 3.30, H-6) was discerned in IV and this was collapsed to a singlet in V; this could be ascribed to originate from a proton at δ ∿7.4 (-CH=, H-6) in I, II and III, because in V this was the only one-proton singlet. By irradiation at H-5 the octet of H-6,6' collapsed to an AB quartet (J 13Hz) indicating the presence of $-CH_2(6,6')-\dot{C}H(5)-$ in IV and $-CDH(6)-\dot{C}D-$ in V, however, the triplet at δ 4.04 (H-2) was proved not to be coupled to any of H-5,6 and 6' but coupled with the protons at δ ∿1.7. The fact that H-2 in IV appeared as a triplet requires the presence of two hydrogens at the vicinal positions of the H-2 and this is satisfied, in this case, only by situating a methylene group to the position. Moreover, considering the no coupling of H-2 with H-5,6 and 6', H-2 is concluded to be produced by the hydrogenation of $-\dot{C}=N-$.
Therefore the following reaction was confirmed:

$$AcNHCH_2CH_2CH_2-\dot{C}=N- \text{ (in III)} \longrightarrow AcNHCH_2CH_2CH_2-\dot{C}H(2)-ND- \text{ (in IV in } D_2O)$$

At the same time, the following reaction was also confirmed:

$$-CH(6)=\dot{C}(5)-NH_2 \text{ (in III)} \longrightarrow -CH_2(6,6')-\dot{C}H(5)-NH_2 \text{ (in IV)}$$

Since, as mentioned above, H-2 in IV did not couple with H-5 and H-6,6', and H-6,6' coupled only with H-5, the two parts could be combined as $AcNHCH_2CH_2CH_2-\dot{C}H(2)-NH-CH_2(6,6')-\dot{C}H(5)-NH_2$.
The way of attachment of the above chain to another part $-\underset{|}{C}OCH(4)-CH(CH_3)_2$ was solved by the fact that H-4 and H-5 were coupled with each other and that H-2 was coupled with a methylene group only.

From the aforementioned results, the structure of arglecin can be concluded to be 5-amino-2-(3-guanidinopropyl)-3,4-dihydropyridin-3-one (I). The structure is reasonable from the biosynthetic viewpoint because it can be constructed from arginine and leucine. It is noteworthy that this compound has been found in the culture filtrates of about thirteen *Streptomyces* species.

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