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SYNTHESIS OF PYRAZOMYCIN

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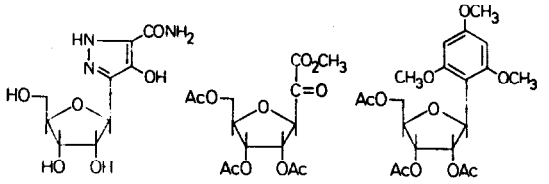
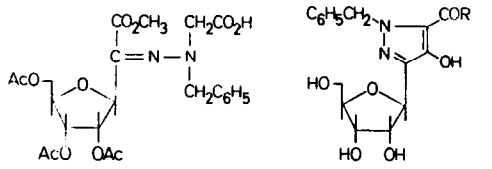
We wish to report the synthesis of a nucleoside antibiotic, pyrazomycin[1] (I) using a procedure suggested previously[2] as a general route to 3-substituted 4-hydroxypyrazole-5-carboxylic acids.



I II III

We started from the α-keto acid ester II which was obtained, as reported earlier[3], by ozonolytical cleavage of the C-glycoside III.



IV V R=OCH$_3$ VI R=NH$_2$

When treated with (1-benzylhydrazino) acetic acid[4] in a methanolic solution, II was converted to the hydrazone derivative IV which was, in turn, treated with sodium acetate in acetic anhydride at 100°C for 8 hours. The reaction mixture was worked up, treated with an ethereal solution of diazomethane and filtered through a column of silica gel (benzene - acetone 9.5 : 0.5 as eluant) to separate the dark colored impurities. The eluate was subjected to base-catalysed methanolysis to remove the acetyl groups. The obtained mixture was separated by chromatography on a column of silica gel (chloroform - - acetone - methanol 16 : 3 : 1 as eluant). The fractions exhibiting UV spectra characteristic of 4-hydroxypyrazoles were collected and purified by chromatography on a column of Dowex 1 (acetate) ion exchange resin with a linear gradient of acetic acid, to give the product V as a freeze-dried powder in 9.5% yield (based on the starting

compound III). The product V, thus obtained, was homogeneous, as shown by chromatography on a column of Ecteola, using a linear gradient of triethylammonium hydrogen carbonate. The analytical and spectrometrical data of the product V are consistent with the proposed structure. For $C_{17}H_{20}N_2O_7$ (364.4) calcd. 56.04% C, 5.53% H, 7.69% N; found 56.20% C, 5.54% H, 7.89% N. CD spectrum in water: $[\theta]_{276}$ -2590, $[\theta]_{250}$ 0, $[\theta]_{232}$ 2355, $[\theta]_{219}$ 0. UV spectrum: λ max in 0.1 M HCl 234 nm and 276 nm (logε 3.82 and 3.74); λ max in 0.1 M NaOH 320.5 nm (logε 3.94) and inflex at 238 nm (logε 3.75). Mass spectrum: M^+ at m/e 364 and B + 30 at m/e 261. NMR spectrum at 100 MHz in DMSO-d_6, internal tetramethylsilane, δ in ppm: 3.65 (m, 2H, $H_{5'}$), 3.79 (s, 3H, CO_2CH_3), 3.91 (m, 1H, $H_{4'}$), 4.10 (t, 1H, $J_{3',2'}$ 5.2 Hz, $J_{3',4'}$ 5.2 Hz, $H_{3'}$), 4.31 (t, 1H, $J_{2',1'}$ 6.0 Hz, $J_{2',3'}$ 5.2 Hz, $H_{2'}$), 4.84 (d, 1H, $J_{1',2'}$ 6.0 Hz, $H_{1'}$), 5.54 (s, 2H, CH_2Ph), 7.0 - 7.35 (m, 5H, C_6H_5).

Treatment of V with methanolic ammonia at 100°C for 8 hours, followed by chromatography on a column of silica gel (in ethyl acetate - acetone - methanol - water 7 : 1 : 0.5 : 0.5) gave the amide VI, obtained as a freeze-dried material in 80% yield. For $C_{16}H_{19}N_3O_6$ (349.3) calcd. 55.01% C, 5.48% H, 12.03% N; found 54.51% C, 5.51% H, 11.83% N. CD spectrum in water: $[\theta]_{268}$ -2590, $[\theta]_{239}$ 0, $[\theta]_{228}$ 368, $[\theta]_{218}$ 0. UV spectrum: λ max in 0.1 M HCl 232 nm and 268 nm (logε 3.92 and 3.82); λ max in 0.1 M NaOH 239 nm and 312 nm (logε 3.77 and 3.92).

Hydrogenolytic debenzylation of V, followed by purification on silica gel (in ethyl acetate - acetone - methanol - water 6 : 1 : 1 : 1 as eluant) and recrystallisation from water, afforded a crystalline product (yield 58%), melting at 112-115°C without depression on admixture with an authentic sample of natural pyrazomycin (I). For $C_9H_{13}N_3O_6$ (259.2) calcd. 41.70% C, 5.06% H, 16.21% N; found 41.69% C, 5.16% H, 15.89% N. CD spectrum[5] in water: $[\theta]_{263}$ -3145, $[\theta]_{240}$ 0, $[\theta]_{225}$ 2983, $[\theta]_{217}$ 0. UV spectrum: λ max in 0.1 M HCl 225 nm and 266 nm (logε 3.64 and 3.49); λ max in 0.1 M NaOH 306 nm (logε 3.72) and inflex at 233 nm (logε 3.56). Mass spectrum: M^+ 259 and B + 30 at m/e 156. These analytical and spectrometrical data as well as the infrared spectrum in KBr, and chromatographical and electrophoretical mobilities confirm the identity of the synthetic material with pyrazomycin.

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REFERENCES

1. K. Gerzon, R.H. Williams, M. Hoehn, M. Gorman and D.C. Delong, 2nd International Congress of Heterocyclic Chemistry, Montpellier, France 1969, Abstract C-30.
2. J. Farkaš and Z. Flegelová, Tetrahedron Letters 1971, 1591.
3. L. Kalvoda, J. Farkaš, and F. Šorm, Tetrahedron Letters 1970, 2297.
4. (1-Benzylhydrazino) acetic acid (m.p. 161-163°C) was obtained by electrochemical reduction of N-nitroso-N-benzylglycine.
5. CD spectrum of natural pyrazomycin in water: $[\theta]_{261}$ -3250, $[\theta]_{239}$ 0, $[\theta]_{225}$ 3060, $[\theta]_{216.5}$ 0.