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PREPARATION OF THE N,N-DIMETHYL-p-METHOXY-L-PHENYLALANYL ANALOGUE OF PUROMYCIN

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It has recently been shown that the puromycin derivative, 6-dimethylamino-9-(3'-N, N-dimethyl-p-methoxy-L-phenylalanylamino-3'-deoxy-β-D-ribofuranosyl)-purine (II) is capable of releasing (^{14}C) labelled peptide chains from rat-liver polysomes[1]. Further, the new compound which bears a tertiary amino acid, has no effect on polysome structure as evidenced by sucrose gradient centrifugation. Detailed studies on the fragmentation of the new derivative as well as other puromycin derivatives by mass-spectrometry have recently been reported[2].

In this communication we describe the synthesis of II (hereafter called N,N-dimethyl-puromycin) and some of its properties.

Preparation of N,N-dimethylpuromycin (II) from puromycin .2HCL (I) -
To a solution of puromycin .2HCL (100 mg., 183 μ moles) in 4 ml . of water was added 0.11 ml. of aqueous formaldehyde (1000 μ moles) and 100 mg. of 10% palladium charcoal catalyst[3]. The mixture was then hydrogenated at atmospheric pressure and room tempreture until the theoretical amount of hydrogen had been taken up (7 hr.). The catalyst was extracted with warm water (70°) by four successive extractions (10 ml. each), and the combined

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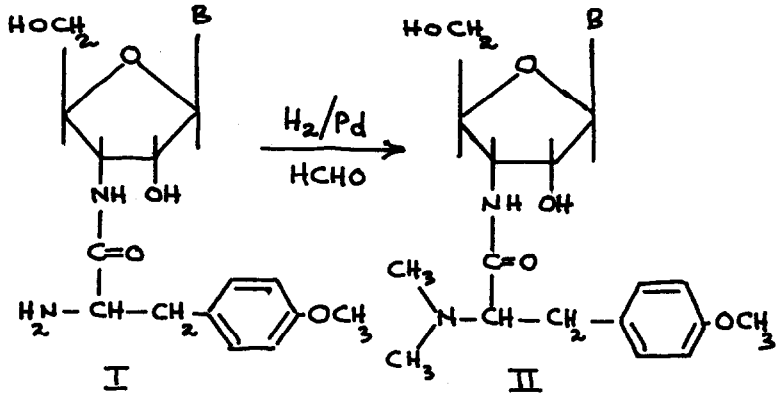
extracts filtered through celite. The resulting clear solution was evaporated to dryness in vacuo at 40°., giving a white residue which was dissolved in absolute ethanol and then again evaporated to dryness. This procedure was repeated twice more. Recrystallisation of the final residue from isopropanol gave small crystalline plates, m.p. 163 - 164.5°. Crystallisation was first allowed to procede at room tempreture, and thereafter at 5° in the refrigerator for a number of hours. Recrystallisation from ethanol was also used, but isopropanol was found to be the more useful solvent.



B = 6 - dimethylaminopurine

The ultraviolet-absorption spectrum showed λ max 269 mμ (in 0.1N-HCL) and λ max 275 mμ (in 0.05 M tris-HCL buffer, pH 7.6). Molecular weight, 499 (mass-spectrometry); theoretical, 499 (as free base). Paper chromatography with solvent A gave an R_F value of 0.72 (puromycin, R_F, 0.65), while on cellulose thin layer chromatography using solvent B it had an R_F value of 0.73 as compared with puromycin, R_F, 0.68. The new compound was unreactive to ninhydrin (0.3% ninhydrin in acetone, heated at 90°C for 15 min.), while puromycin gave a strong positive reaction. Analysis - Found: C, 48.73; H, 6.61; N, 16.61%. $C_{24}H_{33}O_5N_7$ .2 HCL . IH_2O (dihydrochloride monohydrate) requires C,48.80; H, 6.27; N, 16.57%. Infra-red spectra, ν_{max} (KBr) 3240, 3200, 3050, 1684, 1665, 1595, 1560 and 1520 cm^{-1}.

Solvent A: butan-1-ol-acetic acid-water (4:1:5, by vol.)

Solvent B: tertiary amyl alcohol-formic acid-water (3:2:1, by vol.)

Certain hydrogenation experiments failed to give a crystalline product. In these cases, examination of the starting material, puromycin .2HCL, by paper chromatography in solvent A showed it to be contaminated to the extent of 40% by a second ultraviolet absorbing and ninhydrin positive material (R_F, 0.48 which streaked badly to R_F, 0.65); Use of puromycin .2HCL giving one discrete spot on paper or thin layer chromatograms always gave successful results.

N,N - Dimethylpuromycin diacetate - Acetylation of N,N - dimethylpuromycin (3 mg.) was carried out by the addition of a few drops of acetic anhydride in pyridine (0.2 ml.) for 18 hr. at room tempreture in the dark. The reaction mixture was then taken to dryness under vacuo at 40°C, methanol added and the evaporation procedure repeated. This was carried out three times and the final residue dried over P_2O_5. Molecular weight, 583 (mass spectrometry), theoretical, 583; (free base).

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