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ON THE USE OF METHANOL AS A METHYLATING AGENT

A NEW AND CONVENIENT METHYLATION OF THYMIDINE

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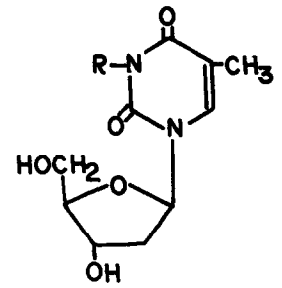
We wish to report on the methylation of the N^3 position of thymidine (I) by methanol in the presence of dicyclohexylcarbodiimide to form N^3-methylthymidine (2'-deoxy-3,5-dimethyluridine) (II). The mechanism of this reaction probably involves the formation of a 2-methyldicyclohexylpseudourea adduct followed by an SN2 attack by a thymidine ion; a mechanism similar to that proposed by Bach (1) for the reaction of ethanol with phenol. This methylation reaction is under further study in an attempt to formulate it on a more general basis. These results will be detailed at a later date.



I R = H
II R = CH_3

The methylation of thymidine with diazomethane (2) or with dimethylsulfate when thymidine is protected (3) has been described. The present method affords a new and convenient approach for the synthesis of this compound and extends the utility of methanol as a methylating agent.

Thymidine (242 mg, 1.0 mmole), DCC (1030 mg, 5.0 mmoles) and abs. methanol (4 ml) were incubated in a sealed tube at 80°-85° for 20 hr. Within a few hours, with occasional shaking, all the thymidine dissolved to form a clear solution. At the end of this time, the reaction mixture was left to cool at room temperature for 1-2 hrs. The crystalline material which formed

was removed by centrifugation and washed with a small volume of 95% ethanol. The combined supernatant fluid and washings were diluted with two volumes of water and the resulting milky solution extracted several times with petroleum ether (B. P. 30°-60°C) until the aqueous layer became clear. The aqueous fraction was separated from insoluble material and concentrated to dryness. The residue was extracted with three 10 ml portions of water and the aqueous extracts were passed consecutively through a 0.78 cm^2 x 3 cm column of Dowex-50W x 8 (H^+ form, 200-400 mesh). The combined colorless effluent was allowed to pass through a 0.78 cm^2 x 2 cm column of Dowex-1 x 8 resin (acetate form, 200-400 mesh). This effluent was concentrated to 2 ml. Upon standing at room temperature for a few minutes, analytically pure N^3-methylthymidine crystallized as a solid mass of needles. After the mother liquor was removed by suction, the crystals were washed with a small amount of cold water, (0.5 ml) and dried in vacuo at 100° for 2 hr. N^3-Methylthymidine sinters at 131° and melts at 134°, micro m.p., K. Upon concentration of the mother liquor, an additional amount of derivative was obtained. Total yield 114 mg (44.5%) of chromatographically pure compound. Analysis calculated for $C_{11}H_{16}N_2O_5$: C, 51.56; H, 6.29; N, 10.93. Found: C, 51.57; H, 6.54; N, 11.06.

For reference purposes N^3-methylthymidine, synthesized by the method of Miles (2a), was purified on ion exchange resins as described above and dried at 100° in vacuo. The N^3-methylthymidine, synthesized by the present method and by the methods of Miles (2a), had identical melting points and mixed melting point (134°). Their infra red spectra in KBr were also identical. Experiments performed to establish optimal conditions indicate that traces of water do not effect the reaction. When the ratio of DCC to thymidine is 1.0, the reaction does not occur (yield of N^3-methylthymidine = 1%). When the ratio of DCC to thymidine is 2.0 or 3.0, the reaction mixture contains 10% and 5% of unreacted thymidine respectively. If the temperature is raised to 90° at these lower DCC to thymidine ratios, the amount of unreacted thymidine becomes 5% and 0% respectively. The optimal yield of II (46%) was obtained at T = 85° and in the presence of freshly distilled DCC.

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