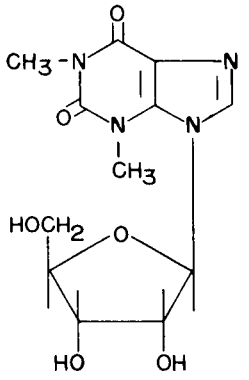
Department of Chemistry, Arizona State University

The Characterization of 1,3-Dimethylxanthosine (9-β-D-Ribofuranosyltheophylline)

Arthur D. Broom and Roland K. Robins (1)

Sir:

1,3-Dimethylxanthosine (I) occupies a prominent place in the classical structure proof of the naturally occurring purine nucleosides. Levene (2) claimed to have obtained I by the action of diazomethane on xanthosine, thus eliminating positions 1 and 3 as possible sites for attachment of the D-ribose moiety. Levene and Sobotka (3) prepared synthetic "theophylline riboside" from acetobromoribose and silver theophylline and claimed the synthetic product to be identical to 1,3-dimethylxanthosine (I) obtained from xanthosine, based on a comparison of optical rotation and rates of acid hydrolysis (3). Gulland and coworkers (4) point out that these two nucleosides could not possibly be identical since the bromotri-*O*-acetylribose used by Levene and Sobotka (3) possessed the pyranose structure. In addition, the synthetic "theophylline riboside", was shown by ultra-violet absorption spectra (4) of similar synthetic theophylline nucleosides, to be in reality the 7-glycosyl derivative. Attempts by Gulland, Holiday and Macrae (4) to repeat the diazomethane methylation of xanthosine as described by Levene (2) resulted in a complex reaction mixture in which no theophylline was detected by acid hydrolysis. These investigators (4) point out that Levene's product was inadequately characterized and suggest he did not obtain 1,3-dimethylxanthosine. They, therefore, made numerous efforts to methylate xanthosine by other means (4, 5) in a futile attempt to prepare authentic I.



I

Despite this criticism, the 1,3-dimethylxanthosine of Levene (2) has been currently quoted as a valid argument for nucleoside structural assignments (6-9). Disregarding Levene's work, Bredereck, Haas and Martini (10) claim to have prepared 1,3-dimethylxanthosine (I) ($[\alpha]_D^{20}$ + 4.6, H_2O) by the action of dimethylsulfate on xanthosine. The synthesis of I is also claimed by these workers (11) from the action of diazomethane on triacetylxanthosine.

Buhler and Pfleiderer (12) have recently confirmed the results observed in our own laboratory in that the procedures of Bredereck (10, 11) yield a mixture of products, none of which are authentic 1,3-dimethylxanthosine (I). It is quite clear that dimethylsulfate under the conditions employed (10) results in rapid methylation of xanthosine at position 7 followed by opening of the imidazole ring (13, 14). In recognition of the foregoing facts Buhler and Pfleiderer (12) recently claimed the *first* synthesis of a 9-glycosyltheophylline.

In connection with a program of study involving the methylation of purine nucleosides (13, 15), the diazomethane methylation of xanthosine, according to Levene (2), was reinvestigated in our own laboratory. The crude product of Levene (product insoluble in ether from a 10 g. run of xanthosine) was crystallized first from absolute ethanol and then from aqueous ethanol to yield 200 mg. of pure 1,3-dimethylxanthosine (I) (3% yield) m.p. 195-198°, $[\alpha]_D^{24}$ -50.7 (C = 1, H_2O).

Anal. Calcd. for $C_{12}H_{16}N_4O_6$: C, 46.1; H, 5.14; N, 17.95. Found: C, 46.2; H, 5.13; N, 18.15.

This product was found to be homogeneous when examined in three different solvent systems. In 5% aqueous ammonium bicarbonate the R_f was 0.83. In conc. aqueous ammonia-dimethylformamide-isopropanol, 10:25:65 (v/v), the Rf was 0.53. In ethanol-water, 70:30 (v/v) the R_f was 0.68. (Chromatograms developed on Whatman No. 1 by the ascending technique.) The ultraviolet absorption spectrum was found to be very similar to that of isocaffeine (4). λ max (*p*H 1), 240 mμ (ε, 10,300); 267 mμ (ε, 11,200). λ max (*p*H 11), 241 mμ (ε, 12,500); 267.5 mμ (ε, 10,900). λ max (MeOH), 240 mμ (ε, 12,300); 265.5 mμ (ε, 10,400).

Proton magnetic resonance studies of I in D_2O (TMPSS internal standard) showed the presence of a sharp singlet at 3.37 (3H) and another sharp singlet at 3.82 (3H) each due to an N-methyl group. The C_8 proton was noted at 8.10 (1H) in addition to the

usual pattern for the D-ribofuranose moiety. The product was further characterized by mild acidic hydrolysis to theophylline and D-ribose which were identified with the aid of ultraviolet spectra and rigorous chromatographic comparison with authentic samples. It is quite clear that Levene (2) did indeed obtain a small amount of 1,3-dimethylxanthosine (I) although in a rather crude form as evident by the rotation of $[\alpha]_D^{24}$ -28 as originally recorded by Levene (2). The preparation and isolation of I was repeated several times according to Levene's directions to give from 1%-3% yield of pure 1,3-dimethylxanthoxine. It is interesting to note that 3-methylxanthosine has recently been reported (16) by the action of diazomethane on xanthosine in dimethylacetamide.

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