EFFECT OF SELECTIVE ACYLATION ON THE ORAL ABSORPTION OF A NUCLEOSIDE BY HUMANS

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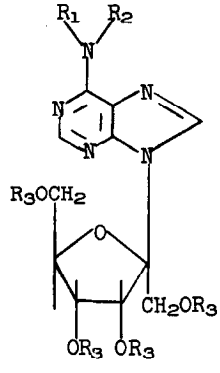
The nucleoside psicofuranine (Eble et al., 1959; Yüntsen, 1958), I, has demonstrated antibacterial efficacy in mice (Lewis, 1959) and antitumor activity in rats upon oral administration (Evans and Gray, 1959). Significant absorption from the gastrointestinal tract of humans failed to occur, as determined by blood level measurements using the microbiological assay of Hanka (1959) and the chemical method of Forist (1959).



I $R_1 = R_2 = R_3 = H$

II $R_1 = R_2 = H:R_3 = Ac$

III $R_1 = H:R_2 = R_3 = Ac$

IV $R_1 = R_2 = R_3 = Ac$

Basicity due to the 6-amino group, and the additional non-lipophillic character ascribed to the four hydroxyl groups are the suspect molecular features which may account for poor absorption (Schanker, 1960). This communication describes the first chemical modification of a nucleoside which dramatically alters its oral absorption pattern in humans.

Results and Discussion

Acetylation of psicofuranine was carried out in the usual manner with acetic anhydride in pyridine at room temperature. By controlling the quantities of the

acetylating agent one could obtain the O-tetraacetate (II) melting 83-86°, $[\alpha]_D$ = -28° (C, 1% ethanol), or a mixture of the pentaacetate (III) and hexaacetate. The latter were readily separable by countercurrent distribution in the system water:95% ethanol:ethylacetate:cyclohexane (w/v 3.0:2.0:2.5:2.5) into crystalline III, melting 113-115° with a distribution coefficient of K = 0.49, and amorphous IV, K = 1.86. Whereas II retained the basic character of the parent nucleoside, III and IV were neutral. Under mild basic conditions II was readily saponified to psicofuranine while III and IV were only converted to their corresponding amides (R_1 = Ac, R_2 = R_3 = H: and R_1 = R_2 = Ac, R_3 = H).

It was of interest then, to note that when the acetates II, III and IV were administered subcutaneously, along with psicofuranine as a control, to mice infected with S. hemolyticus, the tetraacetate demonstrated efficacy equivalent to that of psicofuranine while the neutral acetates were significantly less active (Table I). By the oral route the tetraacetate appeared twice as active as the parent compound. Previous data (unpublished) had shown that psicofuranine was not absorbed by humans. These data, then, suggested the selection of the tetraacetate for clinical trials.

Table I

Relative In Vivo Activities of Psicofuranine Acetates

Compound	CD_{50} Subcutaneous	CD_{50}* Oral
Psicofuranine (I)	6.5	28.1
Psicofuranine Tetraacetate (II)	6.4	11.2
Psicofuranine Pentaacetate (III)	71	
Psicofuranine Hexaacetate (IV)	50-100	

* CD_{50} is the dosage in mg/kg required to produce 50% survivors.

Single dose oral absorption studies of psicofuranine tetraacetate and psicofuranine in humans showed the acetate to be well absorbed while the parent compound

failed to give detectable levels (Fig. 1). All assays were performed on coded samples.

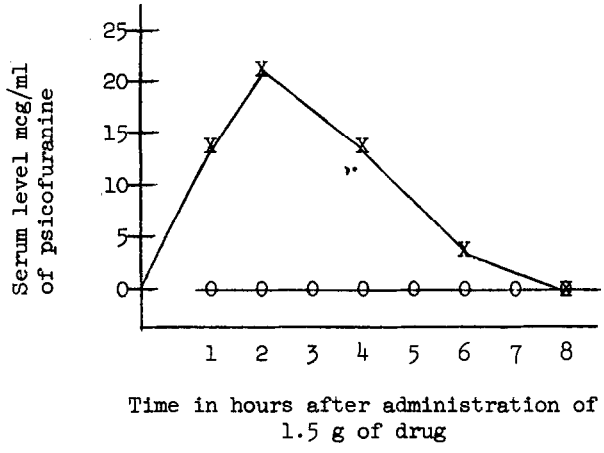


Fig. 1. Human serum levels of psicofuranine and psicofuranine tetraacetate, orally administered.

—X—X—X— II, 6 patients

—0—0—0— I, 2 patients

Lack of difference in serum levels determined by assays with or without a preliminary hydrolytic procedure developed by Hanka (unpublished) suggested that tetraacetate was rapidly converted to free psicofuranine either by blood esterases or through an active transport absorption process. Paper chromatography using the system butanol:water (84:16), 16 hrs, descending, substantiated this observation when it was shown that urines of patients receiving the tetraacetate contained psicofuranine only. In this system psicofuranine displays an R_f of 0.1-0.2 while that of tetraacetate is 0.6-0.8.

Although the absorption mechanisms involved are not established, it should be pointed out that changes in the basicity are apparently not involved since each compound is approximately equally basic. The outstanding difference in physical properties between I and II appears to be the greatly increased lipophillic character of the latter as shown by the solubilities in water and chloroform and the distribution coefficients of each drug for this solvent

pair (Table II). It would appear then that this enhancement in lipophillic character is responsible for the increased absorption.

Table II

Solubilities of Psicofuranine and Psicofuranine Tetraacetate in Water and Chloroform

Compound	Solubility in mg/ml		Distribution Coefficient K mg/ml in H_2O / mg/ml in Chloroform
	Water	Chloroform	
Psicofuranine	13	0.007	992
Psicofuranine tetraacetate	3	>150	0.041

Intravenously administered psicofuranine and orally administered psicofuranine tetraacetate (II), notably nontoxic in numerous animal tests, have proven to be toxic (the chief manifestation being pericardial effusion) at the doses required for human antibacterial and antitumor chemotherapy (Costa and Holland, 1960). However the alteration of the absorption pattern by conversion to the tetraacetate remains of interest.

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