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Biosynthesis of Deuterated Benzylpenicillins III: Relative Antibiotic Potency of Highly Deuterated Benzylpenicillin

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Abstract ☐ The relative antibiotic potency of a highly deuterated benzylpenicillin and [1]H-benzylpenicillin was compared using the official cup-plate bioassay, with *Sarcina lutea* as the test organism. A relative potency (H/D) of 1.23 was obtained. Although the penicillin analogs act by the same mechanism, deuterium apparently affects the potency in an adverse way.

Keyphrases ☐ Benzylpenicillin, highly deuterated—potency compared to nondeuterated benzylpenicillin, cup-plate bioassay, *Sarcina lutea* ☐ Penicillin analogs—effect of high deuteration on potency of benzylpenicillin ☐ Antibiotics—potency of highly deuterated benzylpenicillin, compared to [1]H-benzylpenicillin ☐ Deuteration—effect on potency of benzylpenicillin

The participation of solvent deuterium oxide in the biosynthesis of benzylpenicillin (1) and the isolation and characterization of a highly deuterated benzylpenicillin have been reported (2). Proton magnetic resonance spectra revealed an average replacement of 89% of the protons (^{1}H) with deuterium (^{2}H) atoms. Complete replacement by ^{2}H is observed in the phenylacetyl group, the C-3 position of the thiazolidine ring, and the C-6 position of the β-lactam ring. Partial substitution is noted in the C-5 position of the β-lactam ring (64%) and in the methyl groups (77%) at the C-2 position of the thiazolidine ring.

In the present study, the relative antibiotic potency of this highly deuterated benzylpenicillin and [1]H-benzylpenicillin is compared.

EXPERIMENTAL

Highly Deuterated Benzylpenicillin—Isolation, identification, and characterization of a highly deuterated benzylpenicillin was described earlier (2).

Assay—USP XVII (3) describes a relative potency assay which is useful for determining benzylpenicillin activity as compared to a standard. The cup-plate bioassay, involving *Sarcina lutea* (ATCC 9341) as the test organism (1), was used for this study. Penicillin concentrations are expressed in moles rather than in units per milli-liter or milligrams per milliliter. Expression of the concentrations on a weight basis would introduce a factor involving the difference in molecular weights and would influence the slope in a dose-response relationship (4).

RESULTS AND DISCUSSION

Figure 1 shows the regression lines calculated for highly deuterated potassium benzylpenicillin (D) and potassium [1]H-benzylpenicillin (H) when the results of the assay were represented as inhibition zone diameters (millimeters) on the Y-axis (random variable) and the antibiotic concentration was expressed as log moles $\times$ 10[11] on the X-axis. The statistical methods used by Laskar and Mrtek (4) for their comparisons were utilized here, and a summary of the results is presented in Table I. There was an observed difference in the regression coefficients for the two penicillins, and it was necessary to test the significance. The null hypothesis stated that there was no difference between the slopes of the lines. The results of the slope test were that $t = 0.1625$ (76 df) (not significant), the null hypothesis was accepted, and the parallelism of the slopes was retained. A further test on the regression lines must establish the fact that, although the lines were parallel, intercepts for the two lines were not identical. This test arises from the fact that the intercepts may describe the same locus, and the null hypothesis then was that both regression lines describe the same locus. Application of an identity test reveals that $t = 12.90$ (77 df) ($p < 0.001$) and the null hypothesis can be rejected, indicating that the lines are not identical.

Horizontal displacement may be used to give the relative potency of the [1]H- and highly deuterated benzylpenicillins. This calculation revealed a relative potency (H/D) of 1.23 (95% confidence interval, 1.20–1.26) and indicated that, although the analogs presumably work by the same mechanism of action, deuterium in the molecule affected the potency adversely. Laskar and Mrtek (4) found a relative potency of 1.25 (H/D) for deuteriobenzyl-d_7-penicillin; but a comparison, although tempting, between the results of these workers and the present study would be only cursory since two methods of assay (turbidimetric and cup plate, respectively), two organisms (*Staphylococcus aureus* and *S. lutea*, respectively), and two salts (*N*-ethylpiperidine and potassium, respectively) were used.

Interpretation of Assay Results—A review of the effects of deuterium on therapeutic compounds was presented by Katz and Crespi (5). Of particular interest are the effects of deuterium substitution on the activity of other antibiotic compounds. Nona *et al.* (6) isolated [2]H-griseofulvin and evaluated its *in vitro* activity. The fully deuterated analog was found to be slightly more potent than
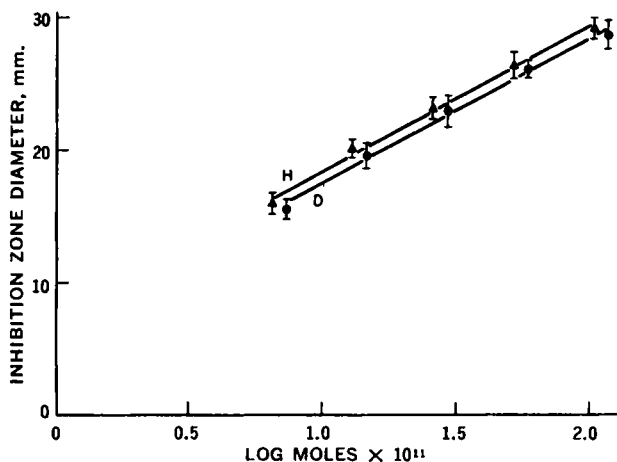
Figure 1—*Regression lines calculated for potassium* [1]*H-benzyl-penicillin* (H) *and highly deuterated potassium benzylpenicillin* (D).

Table I—Calculation Results for Regression Analysis of [1]H- and Highly Deuterated Benzylpenicillin

	Protio	Highly Deuterated
N	40	40
Σy^2	1279.519	1141.624
Σx^2	7.251	7.246
b_{yz} (regression coefficient)	10.838	10.875
F_{ratio} (nonzero slope)	70.28 ($p < 0.001$)	107.36 ($p < 0.001$)
$\bar{X}$ (log moles $\times 10^{11}$)	1.417	1.473
$\bar{Y}$ (mm.)	22.96	22.59
$\hat{Y}_{byz}$ (regression line)	$10.838X + 7.60$	$10.875X + 6.57$
$SS_{dev.\ from\ regression}$	3.99	4.702

the protio analog, but no difference in activity was found between partially deuterated and [1]H-griseofulvins. Laskar and Mrtek (4) synthesized deuteriobenzyl-d_7-penicillin and compared the biological activity of the N-ethylpiperidine salts of the protio- and deuterio-analogs; an H/D ratio of 1.25, indicating a decrease in potency of the deuterio-analog, was observed. Kutter and Machleidt (7) prepared α-deuteriochloramphenicol and compared relative potencies against *Escherichia coli*. A decreased activity of the deuterium-substituted antibiotic was reported.

Penicillin acts by inhibiting cell wall formation, but the exact mechanism of this inhibition is not well understood. Although Collins and Richmond (8) showed a similarity between N-acetyl-muramic acid and penicillin, Wise and Park (9) concluded that, since penicillin did not prevent formation of a polysaccharide back-bone in a staphylococcus *in vitro* system, the N-acetylmuramic acid component was not involved and that penicillin probably acted at the cross-linking level in mucopeptide synthesis. They showed that the penicillin molecule fits the active site of a hypothetical trans-peptidase. The β-lactam ring of the penicillin molecule presumably reacts specifically with the enzyme, and inactivation of the enzyme results. Tipper and Strominger (10) further postulated that penicillin has a configuration like that which is normally at the end of the acetylmuramylpentapeptide fragment. When the penicillin is fixed to the substrate binding site of the transpeptidase, the β-lactam ring opens and a penicilloyl enzyme, which is inactive, is formed. Penicillin has been shown (11) to be irreversibly bound to a "peni-cillin-binding component" of bacterial cell walls, and this compo-nent is presumably the enzyme transpeptidase.

Elison *et al.* (12) reported that the substitution of ^{2}H for ^{1}H in the N—CH$_3$ of morphine resulted in a weaker binding of the deuterio-analog to the N-demethylating enzyme. Hattori *et al.* (13) showed that the introduction of ^{2}H in nonexchangeable ^{1}H positions of protein phycocyanin appeared to decrease nonpolar side-chain interactions. Fisher and Jardetzky (14) and Katz and Crespi (5) observed that the phenyl group was the portion of the benzylpeni-cillin molecule involved in binding to serum protein albumin. If the phenyl group assists in the binding of benzylpenicillin to the enzyme, a decrease in the binding strength of highly deuterated benzyl-penicillin when compared to [1]H-benzylpenicillin would be expected. The acylation of the highly deuterated benzylpenicillin to the enzyme would be slowed, resulting in a decreased potency of the highly deuterated benzylpenicillin. This result, taken with the

result of Laskar and Mrtek (4), indicates that deuteration of any site of penicillin has only a small effect on the biological potency.

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