

The results presented in this paper serve to further demonstrate that simply fortifying samples in the laboratory does not always yield valid recovery data, particularly for biologically incorporated organic compounds. This is especially true for compounds of a polar nature which are usually excreted in urine as conjugates.

SUMMARY

Detailed methodology for the determination of pentachlorophenol in urine has been presented. A hydrolysis time of 1 h was determined to be essential for the determination of both free and conjugated PCP. An acid alumina column cleanup allowed levels of 1 ppb PCP to be detected quantitatively in urine. Recoveries of PCP from urine at fortification levels of 5 ppb and greater averaged better than 90%.

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Epimerization of α -(1-Carboxyethyl) Hydrogen Benzylpenicilloate

α -(1-Carboxyethyl) hydrogen benzylpenicilloate (III), a material formed by the interaction of lactic acid with benzylpenicillin (I) in meat, isomerizes when the meat is heated. The 5-epimer (IV) and, to a lesser extent, the 6-epimer (V) are formed.

In a study to determine the fate of benzylpenicillin (I) residues in meat, DePaolis et al. (1977) found that the major product formed upon freezer storage (-2°C) was α -(1-carboxyethyl) hydrogen benzylpenicilloate (III, Figure 1). Cooking the meat resulted in the formation of larger amounts of III as well as formation of at least one of its isomers. This paper reports the efforts to isolate and determine the stereochemistry of the cooking product(s).

TLC analysis of the cooked meat extract revealed, in addition to benzylpenicilloic and benzylpenilloic acids, the presence of III at R_f 0.45 and a material at R_f 0.32. The quantity of material at R_f 0.32 increased at either higher temperature or with longer cooking times. Attempts to remove the material at R_f 0.32 with subsequent esterification by diazomethane were complicated by the fact that we could not completely rid the system of acetic acid, which had to be used in the eluant mixture and which preferentially reacted with diazomethane. Instead, the materials present in meat were extracted with methanol and eventually converted to their methyl esters with diazomethane as described by DePaolis et al. (1977). TLC separation of this mixture on silica gel (benzene-ethyl acetate, 1:1) gave, in addition to dimethyl benzylpenicilloate (R_f 0.62) and methyl benzylpenilloate isomers (R_f 0.28 and 0.18), material at R_f 0.69 (dimethyl ester of III), 0.60, and 0.56. The latter two both gave essentially similar chemical ionization spectra as III, indicating that the three compounds were isomers. Under "normal" cooking conditions there appeared to be more R_f 0.60 material than material with R_f 0.56, making the former the compound

of greater practical importance.

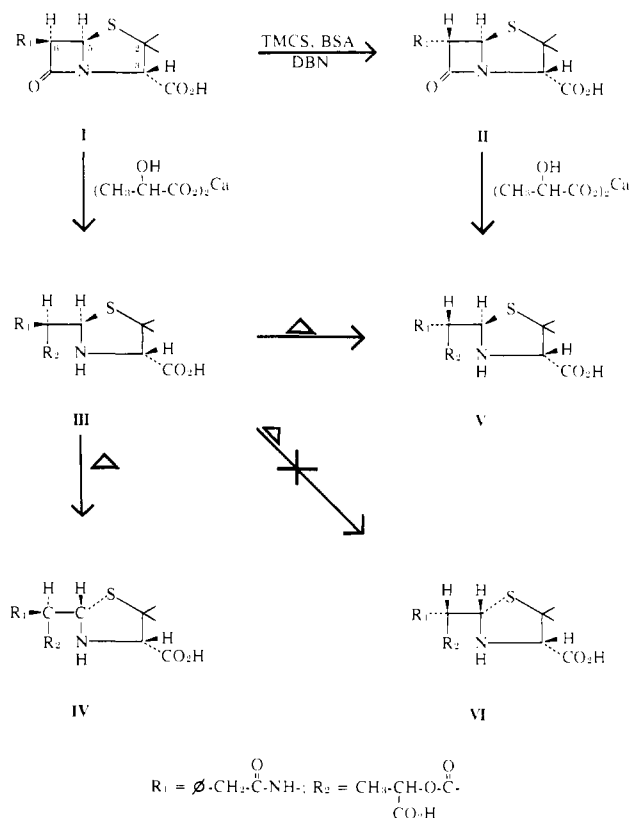
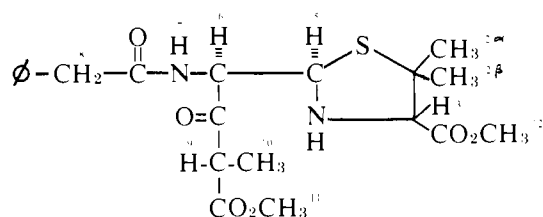
The same materials could be formed by heating III in an oil bath at 80 - 100°C for 30 min. These conditions also promoted the conversion of benzylpenicilloic acid to benzylpenilloic acid. This was very helpful because dimethyl benzylpenicilloate had an R_f value very close to one of the materials of interest in our TLC solvent system.

Material III contains four asymmetric carbon atoms, with epimerization most likely to occur at C-5 and C-6. Epimerization at C-6 in penicillins has been well documented (Kaiser and Kukulja, 1972). The different epimeric forms of benzylpenicilloates have been reported but only the stereochemistry of the epimer corresponding to naturally formed benzylpenicillin has been established with certainty (Sheehan and Cruickshank, 1956). For III, there are three other possible C-5, C-6 epimers (IV, V, and VI, Figure 1). One of these, V, was synthesized by reacting 6-*epi*-benzylpenicillin (II) with an equimolar amount of calcium lactate in dimethyl sulfoxide (Me_2SO) as described earlier (DePaolis et al., 1977) except that 100% Me_2SO was used instead of 50% Me_2SO -water. The change to 100% Me_2SO was necessitated by the fact that II reacted with calcium lactate at a slower rate than I. Conversion of the latter to II was achieved by treatment of I with trimethylchlorosilane (TMCS) and *N,O*-bis(trimethylsilyl)acetamide (BSA), followed by treatment with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) as described by Vlietinck et al. (1973). The epimerization of benzylpenicillin to its 6-epimer could be monitored and quantitated by high-pressure liquid chromatography. The two

Table I. Chemical Shifts (ppm) and *J* Values (cps) for Protons of α -(1-Carboxyethyl) Hydrogen Benzylpenicilloate Dimethyl Ester Epimers

proton no. ^a	spin-spin multiplet	δ (J)		
		III	IV	V
2 α	singlet	1.17	1.13	1.15
2 β	singlet	1.43	1.58	1.31
3	singlet	3.27	3.59	3.66
5	doublet	5.27 (2.8)	4.98 ^b	5.27 ^b
6	2 doublets	4.65 (2.8, 6.2)	4.17 ^c (7.2)	5.17 ^c (8.7)
7	doublet	6.18 (6.2)	6.72 (7.2)	6.24 (8.7)
8	singlet	3.64	3.69	3.69
9	quartet	5.15 (7.1)	5.10 (7.1)	5.22 (7.1)
10	doublet	1.56 (7.1)	1.47 (7.1)	1.46 (7.1)
11,12	singlets	3.72, 3.73	3.71, 3.73	3.72, 3.73
phenyl	multiplet	7.21-7.31	7.20-7.32	7.26-7.37

^a See structure in Figure 2. ^b Singlet. ^c Only one doublet.

**Figure 1.** Structures of materials discussed in the text.**Figure 2.** Proton numbering sequence used in Table I.

isomers were separated by using a Partisol-10 SAX column and sodium acetate (0.25 M, pH 6.7) as eluant at a flow rate of 0.6 mL/min. Under these conditions, I and II had retention times of 6.9 and 7.7 min, respectively.

The TLC R_f value of V on silica gel was 0.29 (ethyl acetate-acetic acid-water, 8:1:1), while the R_f value of its dimethyl ester was 0.56 (benzene-ethyl acetate, 1:1). This gave presumptive evidence for the formation of V during cooking and left structures IV and VI as the only reasonable possibilities for the material which, after treatment with diazomethane, had R_f 0.60 in our TLC system. Comparison (Table I) of the nuclear magnetic resonance (NMR) spectrum (in CDCl_3) of the material with R_f 0.60 to the NMR spectra of the dimethyl esters of III and V indicated that this material was indeed IV.

The H-5, H-6 coupling constant in the cis,cis epimer, III, was 2.8 cps while there was no measurable coupling constant for H-5, H-6 in the cis,trans epimer, V. The material with R_f 0.60 also exhibited no H-5, H-6 coupling constant, a fact consistent with a cis,trans configuration such as depicted in structure IV.

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