J. Pharm. Pharmacol. 1994, 46: 533–534
Received June 14, 1993
Accepted December 23, 1993

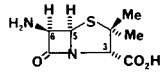
8-Hydroxypenillic acid: NMR characteristics and facile formation from 6-aminopenicillanic acid

A. F. CASY, A. LIPCZYNSKI*, *School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK*

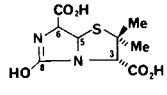
Abstract—High field NMR (^1H and ^{13}C) spectral characteristics in D_2O of 8-hydroxypenillic acid and 6-aminopenicillanic acid (6-APA)-sodium bicarbonate mixtures are reported. The ready conversion of 6-APA to 8-hydroxypenillic acid is demonstrated.

In an analysis of the ^1H NMR features of β-lactam antibiotics (Branch et al 1987), the spectrum of 6-aminopenicillanic acid (*1*, 6-APA) in D_2O solubilized by means of a molar proportion of sodium bicarbonate was reported to be unexpectedly complex. No spectral signals corresponded with those of β-lactam ring-opened products (isomeric penicic acids), and since 6-APA is known to be converted to 8-hydroxypenillic acid (*2*) by exposure to a large excess of carbon dioxide (Ballio et al 1961; Batchelor et al 1961; Johnson & Hardcastle 1961) spectral complexity was attributed to the presence of the penillic acid. This proposal has now been confirmed by a high field ^1H and ^{13}C NMR study of 6-APA-$NaHCO_3$ mixtures and authentic 8-hydroxypenillic acid prepared by the procedure of Johnson & Hardcastle (1961).

Spectral analysis of the ^1H (Table 1) and ^{13}C (Table 2) NMR features of 8-hydroxypenillic acid (isolated as the disodium salt



1. 6-APA



2. 8-Hydroxypenillic acid

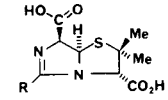
Table 1. ^1H NMR chemical shifts of 8-hydroxypenillic acid, a 6-APA-$NaHCO_3$ equi-molar mixture, and some related standards in D_2O[a].

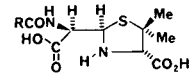
Item	Sample	5-H, 6-H	3-H	2-Me (α, β)
1	8-Hydroxypenillic acid (*2*) 2Na salt	5·52 d(1·96) 4·21 d(1·96)	4·24 s	1·56 s 1·50 s
2	8-Benzylpenillic acid in D_2O-$NaHCO_3$ 100 MHz[b]	5·76 d(4·5) 4·62 d(4·5)	4·16 s	1·49 s 1·47 s
3	6-APA-$NaHCO_3$ (1·1 molar proportion)	Major signals 5·52 d(1·95) 4·21 d(1·95) Minor signals 5·51 d(4·0) 4·65 d(4·0)	4·23 s 4·19 s	1·55 s 1·50 s 1·62 s 1·52 s
4	6-APA Na salt 60 MHz[c]	5·54 d(4) 4·64 d(4)	4·24 s	1·67 s 1·57 s

[a] Novel data recorded at 400 MHz on a Jeol GX400 spectrometer under conditions previously described (Casy et al 1989); chemical shifts in ppm from TMS (external), separations (Hz) in parentheses, s singlet, d doublet. [b] Branch et al (1987). [c] Green et al (1965).

* Present address: Pfizer Limited, Central Research, Sandwich, Kent CT13 9NJ, UK.

Correspondence: A. F. Casy, 5 Piplar Ground, Bradford-on-Avon BA15 1XF, UK.





R = CH_2Ph

3. Benzylpenillic acid (probable configuration)

4. 3*S*,5*R*,6*R*-Benzylpenicilloic acid

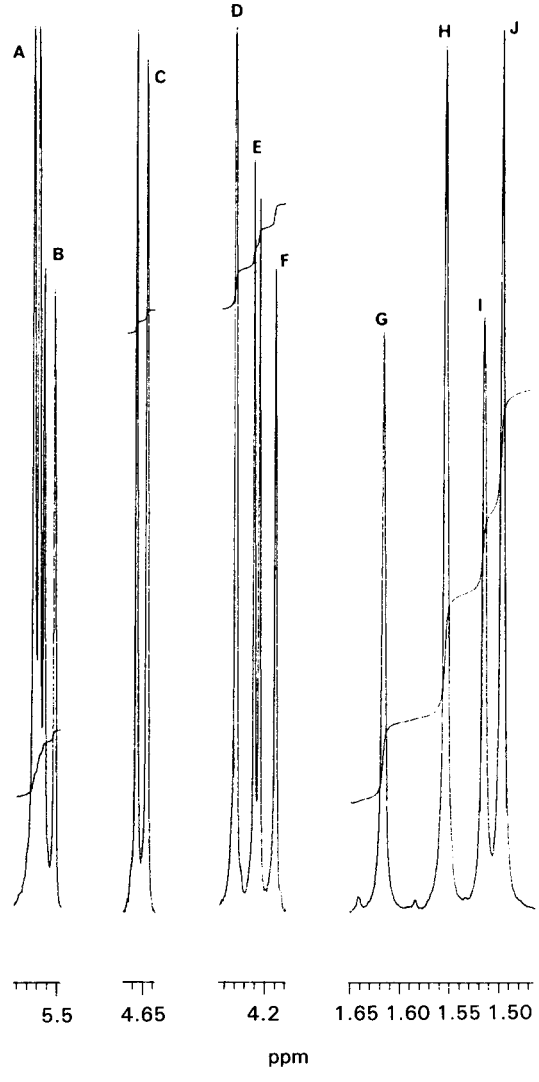


FIG. 1. 400 MHz ^1H NMR spectrum of a 1:1 molar proportion of 6-APA and $NaHCO_3$ in D_2O recorded shortly after dissolution. A.5- or 6-H of *2* (8-hydroxypenillic acid), B.5- or 6-H of *1* (6-APA), C.5- or 6-H of *1*, D.3-H of *2*, E.5- or 6-H of *2*, F.3-H of *1*, G/I.α,β 2-Me of *1*, H/J. α, β 2-Me of *2*.

Table 2. ^{13}C NMR chemical shifts of 8-hydroxypenillic acid, a 6-APA-$NaHCO_3$ equi-molar mixture, and some related standards in D_2O[a].

Item	Sample	C-2	C-3	C-5	C-6	2-Me (α,β)	Carbonyls & C-8
1	8-Hydroxypenillic acid 2Na salt[b]	57·7	59·4	69·3	73·5	25·8 31·5	177·2 175·7 164·2 (C-8)
2	8-Benzylpenillic acid in D_2O-$NaHCO_3$[c]	58·9	69·3	73·4	74·1	27·1 31·0	175·6 174·3 170·2 (C-8)
3	6-APA-$NaHCO_3$ (*1 : 1 molar proportion*)	Major signals					
		57·7	59·4	69·3	73·5	25·8 31·5	177·2 175·7 164·2
		Minor signals					
		68·3	72·5	63·7	60·2	26·4 30·6	174·6 (ionized 3-CO_2H) Lactam CO not resolved
4	6-APA in CD_3OD-TFA[c]	67·1	72·2	65·7	58·9	27·4 31·7	172·5 (3-CO_2H) 170·8 (lactam CO)

[a] Recorded at 67·8 MHz on a Jeol GX 270 spectrometer under conditions previously described (Casy et al 1989); chemical shifts in ppm from TMS (external). The number of protons attached to carbons was established from DEPT experiments. [b] Some assignments are tentative. [c] Branch et al (1986).

by lyophilization) was consistent with the fused imidazoline-thiazolidine structure *2*. Assignment of resonances was aided by the data for benzylpenillic acid (*3*) (Branch et al 1986, 1987) included in Tables 1 and 2 (item 2). Chemical shifts of the 3- and 6-carbonyl carbons (> 170 ppm) gave evidence of the ionized state of both carboxylate functions (Branch et al 1986). The vicinal coupling interaction between the C-5 and -6 protons of *2* was notable for its low value, ~2 Hz compared with 4·5 Hz found for $J_{5,6}$ of benzylpenillic acid. This result is evidence that the two penillic acids differ in configuration at C-5 or C-6—that of benzylpenillic acid is probably 5*R*, 6*R* since its principal degradation product in aqueous solution is 3*S*, 5*R*, 6*R*-benzylpenicilloic acid (*4*) (Lipczynski 1988). The ^1H NMR spectrum of the disodium salt of 8-hydroxypenillic acid was well resolved and displayed no unassigned signals of significant intensity, evidence that the conversion of *1* to *2* proceeds in a highly stereospecific manner.

Item 3 of Tables 1 and 2 gives details of spectra of an approximately equi-molar mixture of 6-APA and $NaHCO_3$, which proved to be binary with half of the signals corresponding with those of 8-hydroxypenillic acid while the remainder related reasonably well to spectral features of the sodium salt of 6-APA (Table 1, item 4). Details of the ^1H NMR spectrum are shown in Fig. 1. The ratio of *2* to *1* in the freshly run spectrum was about 1·5:1 as judged from the 2-methyl integrals. Little spectral change was seen over the next two weeks (apart from the appearance of additional signals of low intensities); however, the spectrum of the sample recorded after six weeks storage revealed 8-hydroxypenillic acid as the preponderant component of the mixture with little evidence of 6-APA.

These results demonstrate the remarkable susceptibility of 6-APA to carbon dioxide and emphasize the need to protect the free acid from contact with atmospheric CO_2 and the avoidance of analytical procedures which involve carbonate adjuncts.

We thank Mr R. Hartell for recording the spectra, and Mr J. Speight for assistance in the synthetic work.

References

Ballio, A., Chain, E. B., Dentice di Accadia, F., Mauri, M. (1961) Identification of a compound related to 6-aminopenicillanic acid, isolated from culture media of *Penicillium chrysogenum*. Nature 191: 909–910

Batchelor, F. R., Gazzad, D., Nayler, J. H. C. (1961) Action of carbon dioxide on 6-aminopenicillanic acid. Nature 191: 910–911

Branch, S. K., Casy, A. F., Lipczynski, A., Ominde, E. M. A. (1986) Carbon-13 NMR of β-lactam antibiotics and related compounds. Magn. Reson. Chem. 24: 465–479

Branch, S. K., Casy, A. F., Ominde, E. M. A. (1987) Application of ^1H nuclear magnetic resonance spectroscopy to the analysis of β-lactam antibiotics and their common degradation products. J. Pharm. Biomed. Anal. 5: 73–103

Casy, A. F., Dewar, G. H., Al-Deeb, O. A. A. (1989) Stereochemical studies of the 4-alkyl-4-arylpiperidine class of opioid ligand. Mag. Reson. Chem. 27: 964–972

Green, G. F. H., Page, J. E., Staniforth, S. E. (1965) Cephalosporanic acids. Part 1. Infrared, absorption and proton magnetic spectra of cephalosporin and penicillin analogues. J. Chem. Soc. 1595–1605

Johnson, D. A., Hardcastle, G. A. Jr. (1961) Reaction of 6-aminopenicillanic acid with carbon dioxide. J. Am. Chem. Soc. 83: 3534–3535

Lipczynski, A. (1988) The Degradation of Benzylpenicillin in Aqueous Solution. PhD Thesis, University of Bath