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Semisynthetic Penicillins

VII.* The Use of Phenacyl 6-Aminopenicillinates in Penicillin Synthesis

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The synthesis of a series of phenacyl 6-aminopenicillinates is described. The suitability of these compounds in the preparation of semisynthetic penicillins is demonstrated by using them as intermediates for preparing pyridylmethylpenicillins which are otherwise difficult to obtain. The antimicrobial activity of these penicillins is shortly described.

For the preparation of penicillins by acylation of 6-aminopenicillanic acid (6-APA), chlorides or mixed anhydrides of the side chain acids are the acylating agents mainly used. They generally react smoothly with 6-APA in aqueous media in the presence of base, or with the triethylamine (TEA) salt of 6-APA in dry organic solvents such as chloroform or methylene chloride.[1]

In certain cases, however, the required acid chlorides or anhydrides are not readily obtained and other types of reactive acyl derivatives must be used. From our experience, the reaction of active esters with 6-APA is often sluggish and low yields are obtained. Coupling the free acids with 6-APA using dicyclohexylcarbodiimide (DCC) also appears to give less favourable results.[2]

In peptide chemistry syntheses with activated esters or coupling reactions using DCC are generally performed with esters of the amino component. In analogy with these reactions we decided to study the acylation of 6-APA esters.

6-APA benzyl ester has recently been used in the preparation of guanidino-substituted penicillins.[3] After the acylation of the ester, the benzyl group was removed by catalytic hydrogenolysis. However, because of poisoning of the catalyst, presumably by the sulphur atom of the penicillin nucleus, this procedure suffers from the disadvantage that relatively large amounts of catalyst are needed. Alkyl esters such as the methyl or ethyl esters of 6-APA[4]

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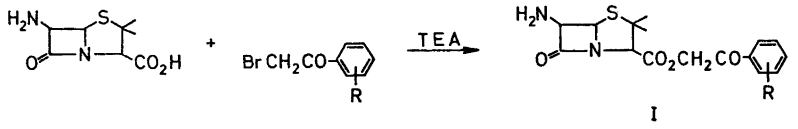
are unsuitable in this connection as they cannot be saponified without extensive hydrolysis of the β-lactam ring.[5,6] 6-APA trialkylsilyl esters show interesting properties in this connection as they can be acylated with various reagents and the trialkylsilyl groups are readily removed.[7] However, because of their extreme sensitivity to hydrolysis, the acylated esters are difficult to obtain in a pure state by extraction procedures.

Since Sheehan and Dave [8] have shown that the phenacyl ester of benzylpenicillin may be cleaved with sodium thiophenoxide in dimethylformamide (DMF) without appreciable destruction of the penicillin nucleus, we decided to try the phenacyl moiety for masking the carboxyl function of 6-APA during the coupling reaction.*

We found that 6-APA phenacyl ester and subtituted phenacyl esters could be readily prepared by treating 6-APA in a mixture of tetrahydrofuran (THF) and dimethylformamide with a suitable phenacyl halide in the presence of an organic base such as TEA.[10]



I

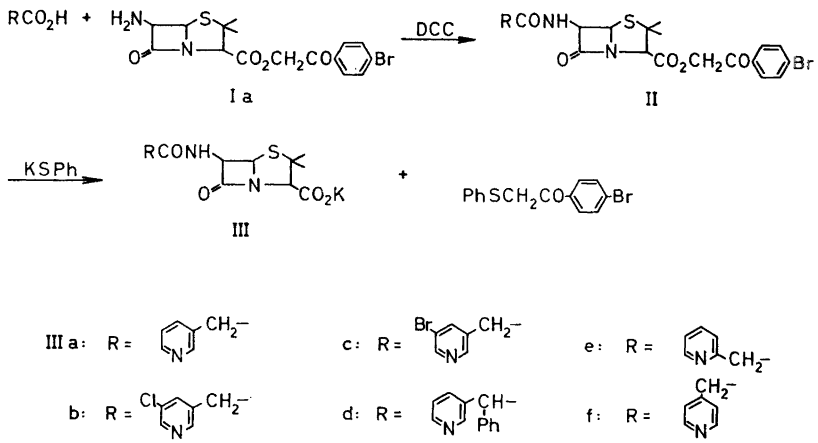
I a: R = p-Br c: R = p-OMe
b: R = H d: R = p-NO_2

The esters were isolated in a crystalline state of high purity as hydrochlorides or as salts of organic sulphonic acids. The hydrochlorides were stable for a few weeks while the latter salts appeared to be stable almost indefinitely at 0—4°C.

The phenacyl esters were used for the preparation of various heterocyclic penicillins and their suitability in penicillin synthesis is demonstrated by the preparation of the pyridylmethyl penicillins (III). Since we found it very difficult to prepare the chlorides or the mixed ethoxyformic anhydrides of the appropriate pyridylacetic acids, they were coupled to the phenacyl esters of 6-APA with the aid of DCC. Of the different esters tried, the *p*-bromophenacyl ester was found to give the best results.

For the preparation of penicillins such as III containing in their side chain basic groups which cannot be masked by protecting groups, the use of 6-APA esters is of especial advantage because the acylated product II is easily purified. If 6-APA itself is used for the preparation of III, the reaction product consists of a mixture of unreacted acid, 6-APA and penicillin, which because of their similar physico-chemical properties, are difficult to separate. Literature data show that in such reactions with 6-APA, low yields are often obtained [11] or tedious extraction procedures must be used to purify the products.[12]

* The same type of reaction has recently been described by workers at Bristol Myers Co., who made use of phenacyl esters in order to prepare penicillins of other types than those reported in this paper.[9]



In the coupling reactions performed in this study, we used a moderate excess of the acylating acid, thus causing all of the 6-APA ester to be acylated. The excess of acid was removed by washing the reaction mixture with aqueous bicarbonate, and the purified penicillin ester thus obtained was treated with potassium thiophenoxide in DMF, yielding the potassium salt of the penicillins. These could often be obtained directly in a crystalline state by adding acetone to the reaction solution.

The penicillins were tested for their stability in acidic media and for penicillinase resistance. Compared to benzylpenicillin, the compounds were found to be about twenty times more stable at pH 2 and slightly more resistant to *Bacillus cereus* penicillinase. The antimicrobial activity was determined in serial dilution tests on agar plates.[13] The activities against *Escherichia coli*, *Staphylococcus aureus*, Oxford, and *Streptococcus faecalis* are presented in Table 1.

Compared to benzylpenicillin, all the compounds appeared to be less active *in vitro* against the two Gram-positive organisms *(Staph. aureus, Strept. faec.)*

Table 1. Purities [15] and minimum inhibitory concentrations [13] of the penicillins.

Penicillin	Purity, %	Activity (μg/ml) *E. coli*	*Staph. aureus*	*Strept. faec.*
III a	100	12.5	0.03	2.5
III b	100	6.25	0.03	1.25
III c	100	6.25	0.03	1.25
III d	73	62.5	0.13	1.25
III e	50	125	0.25	25
III f	63	25	0.06	2.5
Benzyl-penicillin	100	25	≦ 0.006	0.63

whereas III a, b, and c were more active against the Gram-negative organism *(E. coli)*. Among the three unsubstituted 3-pyridylmethylpenicillins (III a, e, f) the 3-isomer proved to be the most active.

EXPERIMENTAL

Those phenacyl bromides which were not commercially available were prepared according to a description given by King and Ostrum.[14] The purities of the penicillins prepared were determined according to the hydroxylamine method, using the potassium salt of penicillin G as standard.[15] For elemental analysis the penicillins in the form of their potassium salts were purified by precipitating them from their aqueous solutions by the addition of acetone.

Paper chromatograms were run in a butanol-ethanol-water (4:1:5, top layer) system and were developed microbiologically.[16] In all cases only one zone corresponding to the penicillin was seen.

p-*Bromophenacyl 6-aminopenicillinate (I a) hydrochloride*. To an ice-cold, stirred suspension of 6-APA (21.6 g, 0.1 mole) and *p*-bromophenacyl bromide (27.8 g, 0.1 mole) in an 1:1 mixture of dry DMF/THF (80 ml), TEA (14 ml, 0.1 mole) was added dropwise during 15 min. The cold reaction mixture was stirred for 3 h and then treated with ethyl acetate (200 ml). The organic phase was washed successively with saturated sodium bicarbonate and sodium chloride solutions. Cold 2 N hydrochloric acid (200 ml) was then added with stirring, yielding a precipitate of the hydrochloride of *p*-bromophenacyl 6-aminopenicillinate. This was collected, and washed with ethyl acetate giving 20 g (45 %) of product. It showed a strong IR absorption band at 1785 cm^{-1}, corresponding to the β-lactam system, and was analytically pure. (Found: C 42.82; H 4.36; Br 17.8; Cl 7.7; N 6.06; O 14.40; S 7.19. Calc. for $C_{16}H_{18}BrClN_2O_4S$ (449.79): C 42.73; H 4.03; Br 17.77; Cl 7.88; N 6.23; O 14.23; S 7.13).

p-*Bromophenacyl 6-aminopenicillinate (I a) benzenesulphonic acid salt*. To an ice-cooled stirred suspension of 6-APA (21.6 g, 0.1 mole) in dry DMF (200 ml), TEA (14 ml, 0.1 mole) was added dropwise during 10 min, followed by addition of *p*-bromophenacyl bromide (28 g, 0.1 mole) in dry THF (200 ml). After stirring for 15 h at 4°C, ethyl acetate (150 ml) and water (250 ml) were added. The aqueous layer was separated and extracted with ethyl acetate (100 ml). The combined organic phases were washed successively with saturated sodium bicarbonate and sodium chloride solutions and dried. Benzenesulphonic acid (8 g) in acetone (100 ml) was added at 0°C giving a crystalline precipitate of the benzenesulphonic acid salt of *p*-bromophenacyl-6-aminopenicillinate. The crystals were collected, washed with acetone and dried, giving 24 g of salt. Addition of benzenesulphonic acid (4 g) in acetone (50 ml) to the mother liquor yielded a further crop of 5 g salt, giving a total yield of 50.8 %. The IR-spectrum of the product exhibited a strong band at 1770 cm^{-1} corresponding to the β-lactam function. (Found: C 46.51; H 4.02; Br 14.16; N 4.75; O 19.42; S 11.03. Calc. for $C_{22}H_{23}BrN_2O_7S_2$ (571.49): C 46.24; H 4.06; Br 13.98; N 4.90; O 19.60; S 11.22).

The following 6-APA esters were prepared in a similar way starting from 6-APA and the appropriate substituted phenacyl bromides.

Phenacyl 6-aminopenicillinate, benzenesulphonic acid salt (I b). Yield 36.8 %, IR absorption at 1780 cm^{-1}. (Found: C 53.60; H 5.08; N 5.50; O 22.62; S 12.91. Calc. for $C_{22}H_{24}N_2O_7S_2$ (492.58): C 53.64; H 4.91; N 5.69; O 22.74; S 13.02).

p-*Methoxyphenacyl 6-aminopenicillinate, benzenesulphonic acid salt (I c)*. Yield 28 %. (Found: C 52.84; H 5.22; N 5.17; O 24.36; S 12.10. Calc. for $C_{23}H_{26}N_2O_8S_2$ (522.61): C 52.86; H 5.02; N 5.36; O 24.49; S 12.27).

p-*Nitrophenacyl 6-aminopenicillinate, benzenesulphonic acid salt*. Yield 20.5 %. IR absorption at 1780 cm^{-1}. (Found: C 48.99; H 4.50; N 7.75; O 26.65; S 12.17. Calc. for $C_{22}H_{23}N_3O_9S_2$ (537.57): C 49.15; H 4.31; N 7.82; O 26.79; S 11.93).

Potassium 3-pyridylmethylpenicillinate (III a). To an ice-cooled stirred suspension of *p*-bromophenacyl-6-aminopenicillinate, benzenesulphonic acid salt (2.4 g, 0.0042 mole) and 3-pyridylacetic acid (0.84 g, 0.0061 mole) in DMF (8 ml), DCC (0.87 g, 0.0042 mole) in DMF (2 ml) was added. After 15 h at 4°C the mixture was poured into an excess of ice-cooled 1 N $NaHCO_3$ and the separated aqueous phase was extracted with ethyl

acetate. The combined organic phases were washed with saturated sodium chloride solution, dried and decolourized on a column of "Aluminium Oxide Woelm neutral, activity grade 1".* After removal of the solvent *in vacuo*, the residue (2.1 g) was dissolved in DMF (6 ml) and treated for 30 min with potassium thiophenoxide (0.45 g, 0.003 mole) in DMF (1.5 ml). Acetone (25 ml) was added, the solvent decanted from the formed precipitate and the product was washed several times with acetone, yielding 1 g (64 %) of the title compound. IR absorption at 1765 cm^{-1}. (Found: C 48.07; H 4.69; K 10.22; N 11.04; S 8.46. Calc. for $C_{15}H_{16}KN_3O_4S$ (373.49): C 48.24; H 4.32; K 10.47; N 11.25; S 8.59).

The following penicillins were prepared in an analogous manner.

Potassium 5-chloropyridyl-3-methylpenicillinate (III b). (Found: C 44.03; H 3.61; Cl 8.51; K 9.61; N 9.59; S 7.52. Calc. for $C_{15}H_{15}ClKN_3O_4S$ (407.94): C 44.17; H 3.71; Cl 8.69; K 9.58; N 10.30; S 7.86). IR absorption at 1765 cm^{-1}.

Potassium 5-bromopyridyl-3-methylpenicillinate (III c). (Found: C 40.36; H 3.51; Br 17.23; K 9.02; N 9.05; S 6.90. Calc. for $C_{15}H_{15}BrKN_3O_4S$ (452.40): C 39.83; H 3.34; Br 17.67; K 8.84; N 9.29; S 7.09). IR absorption at 1765 cm^{-1}.

Potassium α-(3-pyridyl)-benzylpenicillinate (III d). IR absorption at 1750 cm^{-1}; purity 73 %.

Potassium 2-pyridylmethylpenicillinate (III e). IR absorption at 1760 cm^{-1}; purity 50 %.

Potassium 4-pyridylmethylpenicillinate (III f). IR absorption at 1760 cm^{-1}; purity 63 %.

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REFERENCES

1. Doyle, F. P. and Nayler, J. H. C. In Harper, N. J. and Simmonds, A. B., (Eds.), *Advan. Drug Res.* **1** (1964) 21.
2. Hobbs, D. C. and English, A. R. *J. Med. Pharm. Chem.* **4** (1961) 207.
3. Leanza, W. J., Christensen, B. G., Rogers, E. F. and Patchett, A. A. *Nature* **207** (1965) 1395; (Belg. Pat. 634,374/1964).
4. Koe, B. K. *Nature* **195** (1962) 1200.
5. Wintersteiner, O., Boon, W. R., Camington, H. C., MacCorquodale, D. W., Stodola, F. H., Wachtel, J. L., Coghill, R. D., Risser, W. C., Philip, J. E. and Touster, O. In Clarke, H. T., Johnson, J. R. and Robinson, R., (Eds.), *The Chemistry of Penicillin*, Princeton University Press 1949, p. 94.
6. Dürckheimer, W. and Schorr, M. *Ann.* **702** (1967) 163.
7. Sjöberg, B. and Ekström, B. Brit. Pat. 959,853 (1964); Glombitza, K.-W. *Ann.* **673** (1964) 166.
8. Sheehan, J. C. and Daves, Jr., D. G. *J. Org. Chem.* **29** (1964) 2006.
9. Belg. Pat. 684,288 (1967).
10. Bamberg, P., Ekström, B. and Sjöberg, B. Brit. prov. appl. 48208 (1965).
11. Brit. Pat. 1,027,757 (1966); U.S. Pat. 3,296,250 (1966).
12. U.S. Pat. 3,202,653 (1965).
13. Rolinson, G. N., Stevens, S., Batchelor, F. R., Cameron Wood, J. and Chain, E. B. *Lancet* **1960** II 564.
14. King, L. C. and Ostrum, G. K. *J. Org. Chem.* **29** (1964) 3459.
15. Boxer, G. E. and Everett, P. H. *Anal. Chem.* **21** (1949) 670.
16. Batchelor, F. R., Doyle, F. P., Nayler, J. H. C. and Rolinson, G. N. *Nature* **183** (1959) 257.

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