Triphenylpyridinium Derivatives of α-Amino-acids and a Dipeptide

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Primary amines react with the triphenylpyrylium cation in high polarity R_3N–$R'CO_2H$ mixtures and phenolic solvents, forming the usual pyridinium salts. Such reaction of α-amino-acids is accompanied by spontaneous decarboxylation, yielding alkyl substituted *N*-methylpyridinium salts. Pyridinium formation without further decomposition occurs by reaction of the ω-amino- and terminal-amino-substituents of lysine and glycylglycine respectively, these serving as models for amino-residues in polypeptides and proteins.

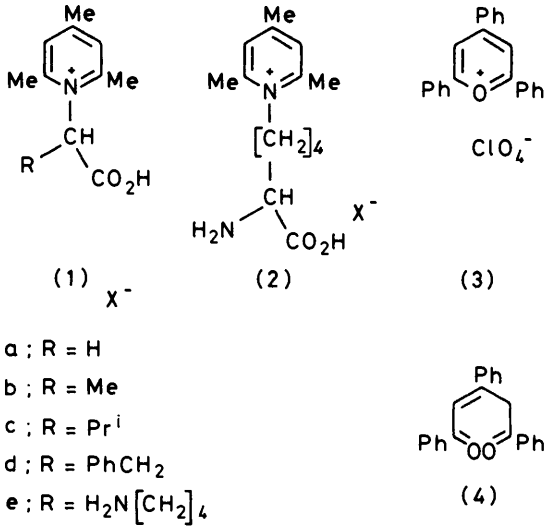
Aryl-substituted pyrylium salts are highly selective reagents for modification of primary amines in the presence of other functional groups: the *N*-alkylpyridinium salts formed undergo substitution at the alkyl carbon α- to nitrogen with many heteroatom or carbon nucleophiles.[1] This method is of obvious potential use for specific replacement of primary amino-substituents in highly functionalised natural products, *e.g.* peptides and proteins.

Reactions of trimethylpyrylium salts in water with α-amino-acids yield pyridinium salts (1a—d):[2-5] different workers claim either of the pyridinium salts (1e)[6] or (2)[4] to be the reaction product of the diamino-acid lysine. Reactions of glycylglycine[5] and the protein chymotrypsin[6] also give trimethylpyridinium derivatives, analogous to (1) or (2). Unfortunately, all such salts (1) and (2) are inert to further useful transformation, due to the very low nucleofugacity of the trimethylpyridine moiety.[1]

Our preliminary attempts to react amino-acids similarly with triphenylpyrylium salts, *e.g.* (3), or condensed analogues, proved unsuccessful, due either to the inactivation of the pyrylium salts by rapid formation in water of insoluble enedione pseudobases, *e.g.* (4), or to the insolubility of the amino-acids in the common organic solvents, *e.g.* CH_2Cl_2, dry EtOH, normally used[1] for arylpyridinium salt formation. We therefore sought non-aqueous solvents of sufficiently high polarity to dissolve amino-acids and peptides. These were first screened for arylpyrylium compatibility by use as media for reactions with common primary amines, and the most suitable then applied to triphenylpyridinium salt formation from amino-acids and peptides.

Preliminary Investigation of Solvents.—Solvents were evaluated by use for the reaction of 2,4,6-triphenylpyrylium perchlorate (3) with n-butylamine, benzylamine, and isopropylamine at 25 °C and comparisons made between the yields of purified pyridinium salts (5a—d) (Table 1). *m*-Cresol gave high yields of pyridiniums (5a,b) and even a satisfactory yield of (5c) from the sterically hindered isopropylamine. Surprisingly, the use of aqueous 90% phenol did not appreciably inhibit pyridinium salt formation, although longer reaction times were required.

A 3 : 2 molar mixture of acetic acid with triethylamine promoted rapid pyridinium salt formation, but yields of (5a,b) were greatly reduced if traces of water were present. Spectroscopic studies showed that even in dry Et_3N–HOAc the pyrylium salt (3) did not dissolve as such, forming instead an acetate adduct (6), $\lambda_{max.}$ 305 nm (log ε 4.32) and δ 7.1—8.0 (15 H, aromatic multiplet) with δ 5.8 and 6.7 1 H singlets for 3-H and 5-H. This adduct (6) evidently reacts satisfactorily with amines to give pyridinium salts, however. The perchlorate (3) was soluble without decomposition in dry Et_3N with CF_3CO_2H (1 : 1) giving the expected n.m.r. spectrum, δ 7.5—8.0 (9 H), 8.4—8.7 (6 H), and 9.2 (2 H, singlet, 3-H and 5-H). Addition of an excess of n-butylamine caused signals due to the pyridinium cation (5a) to develop during 30 min at higher field, *e.g.* a singlet at δ 8.3 due to 3-H and 5-H. However, the preparative reaction in this solvent (Table 1) gave a product contaminated by *ca.* 20% unchanged pyrylium salt, even after prolonged reaction. Such incomplete reactions were not observed in Et_3N–HOAc or the phenolic solvents, which were thus more preparatively useful. Amongst other solvents investigated were (*a*) mixtures of Et_3N with HCO_2H and CCl_3CO_2H, which decomposed; (*b*) mixtures of carboxylic acids with Me_3N, which gave no additional advantages over Et_3N; (*c*) mixtures of phenol with Et_3N (1 : 1), which gave low yields of pyridinium salts.

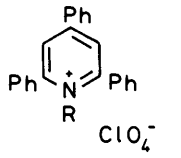


Reaction of α-Amino-acids with Triphenylpyrylium Perchlorate.—Preliminary studies in DMF[7] and EtOH showed that glycine reacted with the pyrylium perchlorate (3) to give the 1-methyl-2,4,6-triphenylpyridinium salt (5e), evidently by spontaneous decarboxylation of the initial product (7). Using CH_3CO_2H–Et_3N as solvent this reaction becomes preparatively useful, giving 1-alkylpyridinium salts (5e—n) in good yield from several amino-acids (Table 2). The ^1H n.m.r. spectra of compounds (5f—n) each showed a singlet at δ 8.1—8.2 (2 H) for pyridinium 3-H and 5-H, an aromatic multiplet at δ 7.1—8.0 [15 H for (5f, g, h, l, m); 20 H for (5i); 19 H for (5j); 21 H for (5k); 18 H for (5n)], and a broad peak at δ

Table 1. Reaction of amines with 2,4,6-triphenylpyrylium perchlorate in high polarity organic solvents

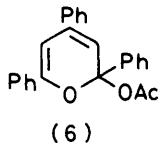
Compd.	Solvent	Amine	Mol equiv.	Method	Reaction time (h)	Pyridinium	Yield (%) [a]	M.p. (°C)
(1)	*m*-Cresol	$PhCH_2NH_2$	1	A	20	(5b)	76	205—207 [b]
(2)	*m*-Cresol	Bu^nNH_2	1	A	72	(5a)	79	206—207 [c]
(3)	*m*-Cresol [d]	Bu^nNH_2	1	B	0.5	(5a)	81	206—207 [c]
(4)	*m*-Cresol	Me_2CHNH_2	2	A	70	(5c)	60	197—199 [e] (decomp.)
(5)	90% Aq. phenol [f]	$PhCH_2NH_2$	1	C	70	(5b)	75	204—206 [b]
(6)	90% Aq. phenol [f]	Bu^nNH_2	1	C	70	(5a)	59	204—206 [c]
(7)	90% Aq. phenol [f]	$PhNH_2$	2	C	360	(5d)	40	258—260 [g]
(9)	AcOH–Et_3N [h,i]	Bu^nNH_2	2	D	0.5	(5a)	78	205—206.5 [c]
(10)	AcOH–Et_3N [h,i,j]	Bu^nNH_2	2	D	0.5	(5a)	68	205—206.5 [c]
(11)	AcOH–Et_3N [h]	$PhCH_2NH_2$	1	D	20	(5b)	25	202—204 [b]
(12)	Phenol–Et_3N [k]	Bu^nNH_2	1	E	70	(5a)	31	204.5—206 [c]
(13)	Phenol–Et_3N [i,k]	Bu^nNH_2	1	E	0.5	(5a)	36	204—205.5 [c]
(14)	CF_3CO_2H–Et_3N	Bu^nNH_2	2	F	20	(5a)	65 [l]	194—198 [c]

[a] Yield of pyridinium salt after recrystallisation from EtOH. [b] Lit., m.p. 205—207 °C (A. R. Katritzky and S. S. Thind, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1895). [c] Lit., m.p. 207—208 °C (A. R. Katritzky and S. S. Thind, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1895. [d] 1 Equiv. of Et_3N added at outset, 2 equiv. of HOAc after 10 min. [e] Found: C, 69.5; H, 5.4; N, 3.1. $C_{26}H_{24}ClNO_4$ requires C, 69.4; H, 5.4; N, 3.1%. [f] Phenol containing 10% water. [g] Lit., m.p. 260 °C (W. Dilthey and H. Dierichs, *J. Prakt. Chem.*, 1935, **144**, 1). [h] Mixture of 3 mol equiv. of AcOH with 2 mol equiv. of Et_3N. [i] Pre-dried by addition of 5% Ac_2O, followed by 2% dry EtOH. [j] Additional AcOH added after 15 min. [k] 1 :1 Molar mixture. [l] Contains *ca.* 20% unchanged pyrylium salt.

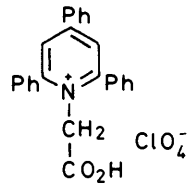


(5)

a ; R = Bu^n
b ; R = $PhCH_2$
c ; R = Me_2CH
d ; R = Ph
e ; R = Me
f ; R = Et
g ; R = Pr^n
h ; R = Me_2CHCH_2
i ; R = $PhCH_2CH_2$
j ; R = *p*-$HOC_6H_4CH_2CH_2$
k ; R = 2-(1*H*-indol-3-yl)ethyl
l ; R = $CH_3CH(OH)CH_2$
m ; R = $HOCH_2CH_2$
n ; R = 2-(1*H*-imidazol-5-yl)ethyl



(6)



(7)

Table 2. Preparation of 1-alkyl-2,4,6-triphenylpyridinium salts from α-amino acids

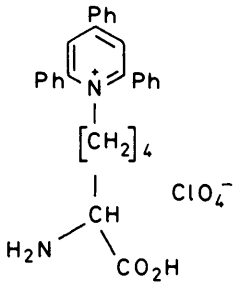
Compd.	Reaction time (h)	Yield [a] (%)	M.p. (°C)	Lit. M.p. (°C)	Lit. Ref.
(5e)	2	81	213—215	214—215.5	*b*
(5f)	2	73	175—177	175—176	*c*
(5g) [d]	2	69	130—132		
(5h) [e]	3	68	237—239		
(5i) [f]	3	58	266—267		
(5j) [g]	3	64	228—230		
(5k) [h]	4	72	203—204		
(5i) [i]	2	65	214—216		
(5m) [j]	2	74	118—119		
(5n) [k]	4	59	215—217		

[a] Yield of pyridinium salt after recrystallisation from EtOH. [b] K. Dimroth, K. Wolf, and H. Kroke, *Liebigs Ann. Chem.*, 1964, **678**, 183. [c] A. R. Katritzky, U. Gruntz, N. Mongelli, and M. C. Rezende, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1953. [d] Found: C, 69.2; H, 5.2; N, 3.0. $C_{26}H_{24}ClNO_4$ requires C, 69.4; H, 5.4; N, 3.1%. [e] Found: C, 69.7; H, 5.7; N, 2.9. $C_{27}H_{26}ClNO_4$ requires C, 69.9; H, 5.7; N, 3.0%. [f] Found: C, 72.6; H, 5.0; N, 2.7. $C_{31}H_{26}ClNO_4$ requires C, 72.7; H, 5.1; N, 2.7%. [g] Found: C, 70.2; H, 4.8; N, 2.6. $C_{31}H_{26}ClNO_5$ requires C, 70.5; H, 5.0; N, 2.6%. [h] Found: C, 71.9; H, 4.8; N, 7.2. $C_{33}H_{26}ClN_2O_4$ requires C, 72.1; H, 4.8; N, 7.1%. [i] Found: C, 66.8; H, 5.5; N, 3.0. $C_{26}H_{24}ClNO_5$ requires C, 67.0; H, 5.2; N, 3.0%. [j] Found: C, 66.1; H, 5.1; N, 3.0. $C_{25}H_{22}ClNO_5$ requires C, 66.4; H, 4.9; N, 3.1%. [k] Found: C, 66.7; H, 4.7; N, 8.1. $C_{28}H_{24}ClN_3O_4$ requires C, 67.0; H, 4.8; N, 8.4%.

4.4—4.5 (2 H) for the methylene group adjacent nitrogen. The spectrum of (5g) also showed signals at δ 1.7 (2 H, β-CH_2) and δ 0.9 (3 H, CH_3); that of (5h) a broad peak at δ 1.8 (1 H, CH) and two singlets centred at δ 1.2 (6 H, two CH_3); and that of (5i, j, k, n) a signal at δ 2.6 (2 H, CH_2 adjacent to the aromatic rings). Compounds (5j, l, m) showed singlets at δ 6.10, δ 3.20, and δ 4.07 (1 H, OH) respectively. Besides, the spectrum of (5l) at δ 4.15 (1 H, CH) and δ 1.13 (3 H, CH_3); and (5m) at δ 4.6 (2 H, CH_2).

Reactions of Lysine and Glycylglycine.—Lysine reacted with the 2,4,6-triphenylpyrylium salt (3) in *m*-cresol in the presence of Et_3N giving a mixture of pyridinium salt and triethylammonium perchlorate from which could be separated some 30% of the pyridinium salt (8). The ^1H n.m.r. spectrum showed the pyridinium 3-H and 5-H at δ 8.1, an aromatic multiplet δ 7—8, and broad peaks centred at δ 4.6, 4.1 and 1.6 (2 H, 1 H and 6 H, respectively) corresponded to lysine ω-CH_2 adjacent to pyridinium nitrogen, unchanged lysine α-CH, and the lysine central trimethylene chain.

The ^{13}C n.m.r. shifts for pyridinium and phenyl ring carbons are similar to values [8] for the 1-butyl-2,4,6-triphenylpyridinium salt (5a). The trimethylene chain gave signals at δ 21.47, 28.25, and 29.31, the acid at 173.5, and the terminal carbon units signals at 53.06 and 54.11 p.p.m., again supporting the



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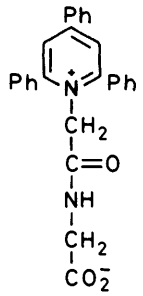
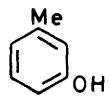


(9)



proposed structure (8), in which the ω-amino-group formed the pyridinium salt. The possible isomeric pyridinium cation formed from the α-amino-group would be expected to give a much lower field signal for the α-carbon, and higher field for the ω-CH_2 group: also decarboxylation could lead to loss of the carbonyl group.

Glycylglycine similarly reacted with triphenylpyrylium cation in the presence of NEt_3 in *m*-cresol, giving the zwitterionic pyridinium salt (9) (44%), which co-crystallised with 1 mol equiv. of cresol and was somewhat hygroscopic. The ^1H n.m.r. spectrum showed the usual aromatic signals plus 2 H signals at δ 5.45 (singlet) and 4.1 (doublet) respectively methylene groups adjacent to pyridinium ring, and NH.

Reaction of lysine and glycylglycine with (3) in Et_3N–CH_3CO_2H and Et_3N–CF_3CO_2H also gave pyridinium salts (8) and (9) identified from their ^1H n.m.r. spectra, but these were more difficult to purify from side-products.

The relatively greater ease of pyridinium salt formation from glycylglycine reflects the greater solubility of this compared with lysine in *m*-cresol. Both results indicate the possibility of similar triphenylpyridinium cation formation both from lysine ω-amino-residues and terminal amino-groups in polypeptides, providing these are adequately soluble in *m*-cresol. An attempt to thus react the pyrylium salt (3) with chymotrypsin was unsuccessful due to inadequate solubility of this large protein in *m*-cresol, or in Et_3N–HOAc.

Experimental

M.p.s were determined using a hot-stage microscope and are uncorrected. Spectra were recorded using a Perkin-Elmer 283B grating spectrophotometer, and a Pye-Unicam SP8-200 u.v. spectrophotometer. ^1H N.m.r. spectra at 60 MHz were recorded using Varian A-60A and EM360, and ^{13}C n.m.r. spectra at 25.5 MHz using a JEOL FX-100 spectrometer.

General Procedure for Reaction of 2,4,6-*Triphenylpyrylium Perchlorate with Amines.*—(A) *In* m-*cresol.* To 2,4,6-triphenylpyrylium perchlorate (1.02 g, 2.5 mmol) in *m*-cresol (5 ml) was added the amine (2.7 mmol) and the red solution kept at 25 °C for the time indicated (see Table 1). Addition of Et_2O (20 ml) gave the pyridinium salt as a white powder, which crystallised from absolute EtOH as needles.

(B) *In* m-*cresol with catalysis.* As for (A), with the addition of Et_3N (0.28 g, 2.8 mmol) at the outset, and CH_3CO_2H (0.36 g, 6 mmol) after 10 min.

(C) *In aqueous* 90% *phenol.* 2,4,6-Triphenylpyrylium perchlorate (1.02 g, 2.5 mmol) was dissolved in phenol containing 10% H_2O (5 ml). The amine (2.5 mmol) was added, and at completion of reaction (see Table 1), the mixture was diluted with water (100 ml), and the precipitate triturated with further water as required; the resulting pyridinium salt was crystallised from absolute EtOH.

(D) *In* Et_3N–CH_3CO_2H. Et_3N (25 g, 0.25 mol) and CH_3CO_2H (22.5 g, 0.375 mol) were mixed, with cooling, to give a homogeneous solution, which was heated at 70 °C with acetic anhydride (0.5 g) for 30 min. EtOH (0.3 ml) was added to give a stock solution of the solvent. In this solution (3 ml) 2,4,6-triphenylpyrylium perchlorate (1.02 g, 2.5 mmol) was treated with the amine (2.8 mmol) at 25 °C for the time specified (see Table 1) with stirring, and the whole diluted with water (200 ml). The precipitated pyridinium salt was crystallised from absolute EtOH.

(E) *In Phenol*–Et_3N. To phenol (2.35 g) and Et_3N (2.5 g) was added Bu^nNH_2 (0.2 g, 2.5 mmol) and 2,4,6-triphenylpyrylium perchlorate (1.02 g, 2.5 mmol). After being stirred at 25 °C for 70 h, the mixture was stirred with water (3 × 100 ml) and the precipitate crystallised from EtOH.

(F) *In* Et_3N–CF_3CO_2H. CF_3CO_2H (800 mg, 7 mmol) and Et_3N (700 mg, 7 mmol) plus $(CF_3CO)_2O$ (80 mg, 0.4 mmol) were kept together for 30 min, when MeOH (10 mg) and 2,4,6-triphenylpyrylium perchlorate (0.51 g, 1.25 mmol) were added, followed by n-butylamine (1 mmol). After 20 h, water (100 ml) was added and the precipitated pyridinium salt crystallised from absolute EtOH.

1-(5-*Amino*-5-*carboxypentyl*)-2,4,6-*triphenylpyridinium Perchlorate* (8).—To 2,4,6-triphenylpyrylium perchlorate (3) (2.04 g, 5 mmol) in *m*-cresol (10 ml) was added lysine (0.8 g, 5.4 mmol) and triethylamine (0.55 g, 5.5 mmol), with stirring, at 25 °C. After 72 h Et_2O (30 ml) was added, and the resulting gum scratched with Et_2O (3 × 50 ml) to give a powder (2 g) which, with acetone (20 ml), gave the *pyridinium* salt (8) (0.74 g, 28%) as microcrystals, m.p. 148—151 °C: it crystallised from EtOH–Et_2O as microprisms, m.p. 160—162 °C (Found: C, 62.5; H, 5.6; N, 5.3. $C_{29}H_{29}ClN_2O_6 \cdot H_2O$ requires C, 62.8; H, 5.6; N, 5.1%); $\nu_{max.}$ ($CHBr_3$) 3 600—2 900, 1 625, 1 600, 1 570, 1 500, and 1 090 cm^{-1}; δ (CF_3CO_2H) 0.8—2.0 (6 H, m), 4.1 (1 H, m), 4.6 (2 H, m), 7.0—8.0 (15 H, m), and 8.1 (2 H, s); δ-(CF_3CO_2H) 21.47, 28.25, 29.31, 53.06, 54.11, 125.95, 128.40, 128.99, 129.40, 130.75, 132.15, 132.80, 133.03, 153.97, 155.73, and 170.35.

1-*Carboxymethylaminoformylmethyl*)-2,4,6-*triphenylpyrylium Salt* (9).—2,4,6-Triphenylpyrylium perchlorate (1.02 g, 2.5 mmol), glycylglycine (0.34 g, 2.5 mmol), Et_3N (0.27 g, 2.7 mmol), and *m*-cresol (5 ml) were stirred at 25 °C for 72 h. Addition of, and washing with ether (3 × 20 ml) and crystallisation from EtOH gave *the zwitterion* (9) (0.60 g, 45%) as prisms, m.p. 197—200 °C (Found: C, 75.5; H, 5.8; N, 5.5. $C_{34}H_{30}N_2O_4 \cdot \frac{1}{2}H_2O$ requires C, 75.7; H, 5.8; N, 5.2%); $\nu_{max.}$ ($CHBr_3$) 3 270, 1 670, 1 625, and 1 580 cm^{-1}; δ (CF_3CO_2H) 2.3 (3 H, s, *m*-cresol), 4.1 (2 H, d, *J* 6 Hz), 5.45 (2 H, s), 6.6—7.3 (4 H, m, *m*-cresol), 7.5—8.0, and 8.2 (2 H, s).

1-*Alkyl*-2,4,6-*triphenylpyridinium Perchlorates* (5e—n).—2,4,6-Triphenylpyrylium perchlorate (1.23 g, 3 mmol) was added to a solvent mixture of Et_3N–CH_3CO_2H (10 ml) prepared as in (D), and treated with the appropriate α-amino-acid (4 mmol). The reaction mixture was stirred at 80 °C for the time specified (see Table 2), cooled, and Et_2O (50 ml) was added. The precipitated pyridinium salt was washed with Et_2O (2 × 10 ml) and crystallised from EtOH.

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References

1 A. R. Katritzky, *Tetrahedron*, 1980, **36**, 679.
2 A. T. Balaban and C. D. Nenitzescu, *Liebigs Ann. Chem.*, 1959, **625**, 74.
3 C. Toma and A. T. Balaban, *Tetrahedron Suppl.*, 1966, **7**, 27.
4 Yu. A. Zhdanov, G. N. Dorofeenko, and A. N. Narkevich, *Zh. Obshch. Khim.*, 1963, **33**, 2418 (*Chem. Abstr.*, 1963, **59**, 14105).
5 A. N. Narkevich, G. N. Dorofeenko, and Yu. A. Zhdanov, *Dokl. Akad. Nauk. S.S.S.R.*, 1967, **176**, 103 (*Chem. Abstr.*, 1968, **68**, 78605).
6 M. H. O'Leary and G. A. Samberg, *J. Am. Chem. Soc.*, 1971, **93**, 3530.
7 B. P. Leddy, Ph.D. Thesis, University of East Anglia, 1977.
8 A. R. Katritzky, R. T. Brownlee, and G. Musumarra, *Tetrahedron*, 1980, **36**, 1643.

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