Potential Chemotherapeutic Agents. Formation of 2,7-Naphthyridones from Nicotinamide Derivatives

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The behaviour of some *N*-benzoylnicotinamide salts with nucleophiles in base is described. In each case addition to C-4 of the pyridine ring occurs, followed by cyclisation to a 2,7-naphthyridone. The reaction with ethyl acetoacetate unexpectedly gave 3-substituted 2,7-naphthyridones.

THE selective inhibition of NAD-dependent enzymatic processes may have chemotherapeutic applications; recently we have prepared some pyridylpyruvic acids which function as inhibitors of lactate dehydrogenase by simulating the normal NAD-substrate transition state.[1] Mechanistic studies suggest that the enzymic conversion of lactate into pyruvate and related reactions involve a hydride transfer from the substrate to the 4-position of the pyridine system of NAD.[2] We considered therefore

[1] D. J. Sheffield and K. R. H. Wooldridge, *J.C.S. Perkin I*, 1972, 2506.
[2] B. Vennesland, *Discuss. Faraday Soc.*, 1955, **20**, 240.

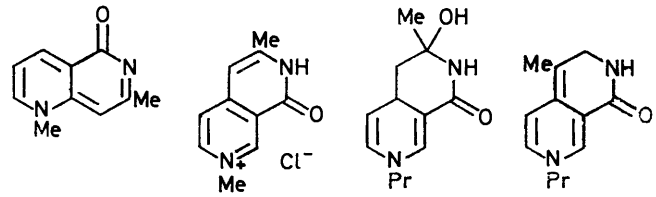
that inhibitors might result from cyclisation of the products of nucleophilic addition to nicotinamide salts.

Nucleophilic addition to pyridinium salts is generally regarded as occurring at the 4-position, but in most cases [3] definite structural proof of the products has not been obtained. Thus, 1,4-adducts have been postulated as the products of addition of cyanide,[4-6] thiols,[7] hydroxylamine,[8] enolate anions [9-11] and imidazole derivatives,[12] primarily on spectral evidence. Huff [13] reported that nicotinamide methochloride and acetone in the presence of base gave a fluorescent compound (1) but later the product was shown by Kröhnke [14] to be the 2,7-naphthyridone (2). Ludowieg proposed on the basis of n.m.r. spectroscopy [15] that two products isolated in low yield from a solution of acetone and 1-n-propylnicotinamide had the structures (3) and (4).

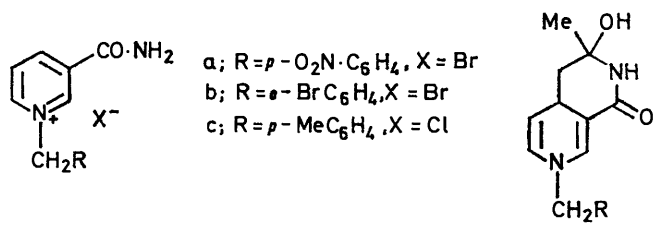
Initially we studied the nucleophilic addition reactions of the nicotinamide salts (5a—c) with acetone in aqueous triethylamine between 5 and 10°. Under these conditions, the nitrobenzyl pyridinium salt (5a) gave a dark brown unstable solid, but salts (5b and c) gave products which were assigned the structures (6b and c) on the basis of the similarity of the n.m.r. spectra to that reported for (3).

Similar reactions of the salts (5a—c) with ethyl cyanoacetate and diethyl malonate gave 20% yields of highly coloured, high-melting solids, the analytical and spectral data of which suggested the formation of 4-substituted 2,7-naphthyridine-1,3-diones (7). Their formation can be rationalised as a nucleophilic attack of the anion at C-4 of (5) followed by elimination of ethanol and dehydrogenation. This constitutes a new synthesis of 4-substituted 2,7-naphthyridine-1,3-diones. In the n.m.r. spectrum of 7-*o*-bromobenzyl-4-cyano-2*H*,7*H*-2,7-naphthyridine-1,3-dione (7; R = *o*-BrC_6H_4, R′ = CN) the C-8 proton signal appears at δ 8·8, finely split by the C-6 proton (*J* 1 Hz), which itself resonates at δ 8·0 and is coupled to the C-5 proton (*J* 6 Hz). This last appears as a sharp doublet centred around δ 7·2. The remainder of the spectrum is fully consistent with the proposed structure.

The action of ethyl acetoacetate on the salts was expected to furnish similar 4-substituted 2,7-naphthyridine-1,3-diones (7; R′ = Ac). However in all cases the analytical and spectral data of the products were not consistent with such structures. For example, elemental analysis of the product from (5c) suggests the molecular formula $C_{20}H_{20}N_2O_3$, this result being supported by the presence of a molecular ion at *m/e* 336 in the mass spectrum. The n.m.r. spectrum shows the presence of an ethyl ester system, and one singlet methyl signal at δ 2·3, indicative of an aryl methyl group. The four aromatic benzyl protons resonate at δ 7·2 and the signals at δ 8·2, 7·35, and 6·5 correspond to the 8-, 6-, and 5-protons of the naphthyridine ring. The one-proton multiplet at δ 11·4, removed upon deuteriation, is the NH signal, and the N·CH_2 singlet appears at δ 5·05. The remaining two one-proton singlets are at δ 5·35 (broad) and 4·48 (sharp). This evidence strongly

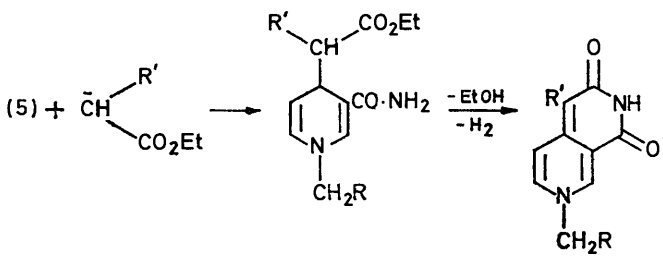


(1) (2) (3) (4)



a; R = *p*-$O_2N\cdot C_6H_4$, X = Br
b; R = *o*-BrC_6H_4, X = Br
c; R = *p*-MeC_6H_4, X = Cl

(5) (6)

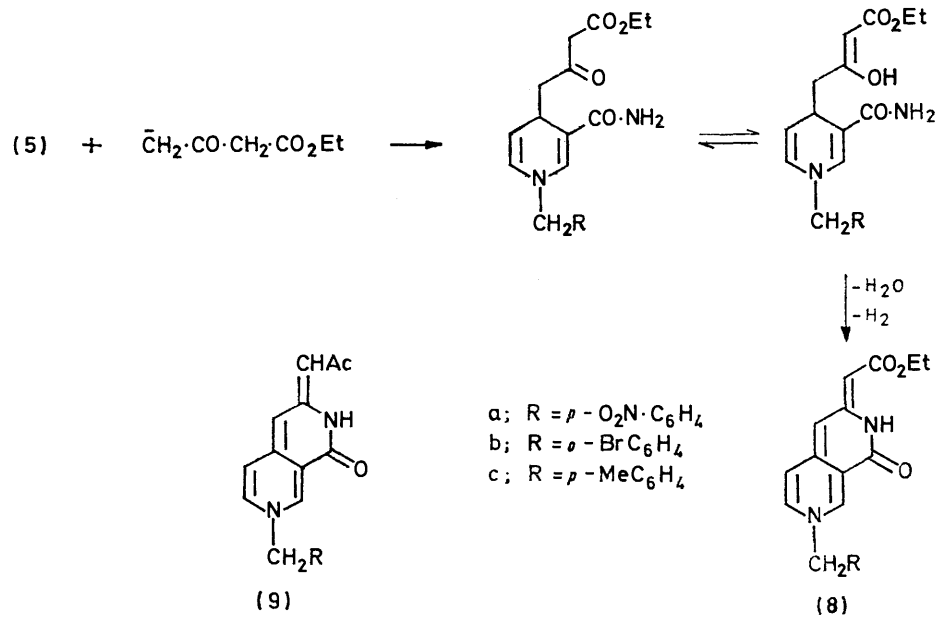


(7)

suggests that the product is 3-ethoxycarbonylmethylene-3,7-dihydro-7-*p*-methylbenzyl-2,7-naphthyridin-1(2*H*)-one (8c). The foregoing singlets correspond to the C-4 proton and the exocyclic methine system, respectively. A possible explanation of this unusual reaction is that proton abstraction by base occurs at the δ-carbon atom of ethyl acetoacetate, followed by nucleophilic attack at C-4 of the pyridinium system, enolisation, and cyclohydration (see Scheme).

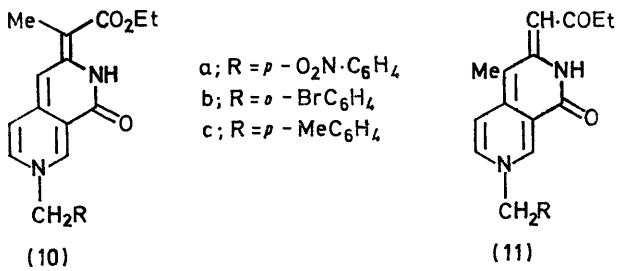
A search for other nucleophiles capable of similar transformation was undertaken. Treatment of the

[3] U. Eisner and J. Kuthan, *Chem. Rev.*, 1972, **72**, 1.
[4] R. N. Linquist and E. H. Cordes, *J. Amer. Chem. Soc.*, 1968, **90**, 1269.
[5] A. G. Anderson and G. Berkelhammer, *J. Org. Chem.*, 1958, **23**, 1109.
[6] H. Diekmann, G. Englert, and K. Wallenfals, *Tetrahedron*, 1964, **20**, 281.
[7] R. M. Burton and N. O. Kaplan, *Arch. Biochem. Biophys.*, 1963, **101**, 139.
[8] R. M. Burton and N. O. Kaplan, *J. Biol. Chem.*, 1954, **211**, 447.
[9] R. M. Burton, A. San Pietro, and N. O. Kaplan, *Arch. Biochem. Biophys.*, 1957, **70**, 87.
[10] R. M. Burton and N. O. Kaplan, *J. Biol. Chem.*, 1954, **206**, 283.
[11] W. Van E. Doering and W. E. McEwen, *J. Amer. Chem. Soc.*, 1951, **73**, 2104.
[12] J. Van Eys, *J. Biol. Chem.*, 1958, **233**, 1203.
[13] J. W. Huff, *J. Biol. Chem.*, 1947, **167**, 151.
[14] F. Kröhnke, K. Ellegast, and E. Bertram, *Annalen*, 1956, **600**, 176.
[15] J. Ludowieg, N. Bhacca, and A. Levy, *Biochem. Biophys. Res. Comm.*, 1964, **14**, 431.



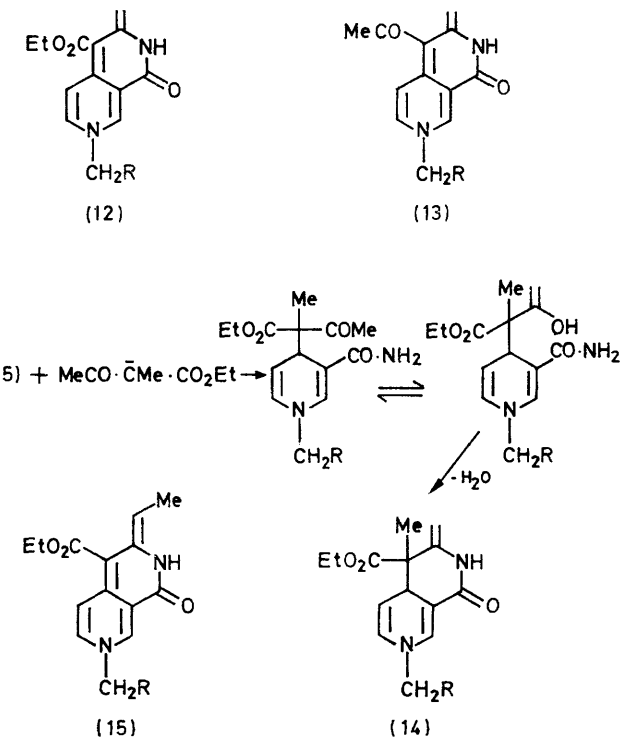
SCHEME

salts (5a—c) with acetylacetone afforded low yields of the 3-acetylmethylene-3,7-dihydro-2,7-naphthyridin-1(2*H*)-ones (9a—c). The spectroscopic data closely resemble those of compounds (8). The sharp singlet attributed to the exocyclic methine proton in the n.m.r. spectrum of (8a—c) occurs further downfield (δ 5·05) in (9a—c), indicating the greater deshielding effect of the acetyl group.



The reaction between the nitrobenzylpyridinium salt (5a) and ethyl α-methylacetoacetate failed to give a stable product. 3-Substituted 2,7-naphthyridones (10b) and (10c) were however obtained with (5b) and (5c), respectively. Analytical and spectroscopic data are in full agreement with these structures. In the n.m.r. spectrum of (10c) the exocyclic methine signal is absent and a three-proton singlet at δ 1·8 (MeC=) is observed. The pyridinium salts (5a—c) reacted with ethyl propionylacetate in a similar fashion to afford compounds (11a—c).

Under the mild basic conditions used in these reactions, one would have expected proton abstraction at the β-carbon atom to occur. Ethyl acetoacetate would then have given the ester (12) and acetylacetone the ketone (13). These compounds would have similar n.m.r. spectra to (8) and (9), respectively, although the exocyclic methylene protons could give rise to two slightly broadened singlets. However reaction of ethyl α-methylacetoacetate at the β-carbon atom would give the naphthyridone (14), which clearly does not fit the n.m.r. data (see Experimental section). Similarly ethyl propionylacetate would furnish (15), which is spectroscopically different from (11).



None of the naphthyridones showed antibacterial, antifungal, or antiprotozoal activity; it is likely that a free amide group is necessary for enzyme inhibition.

EXPERIMENTAL

I.r. spectra were measured on a Unicam SP 200 spectrophotometer for KBr discs. N.m.r. spectra were recorded with a Varian A-60 spectrometer with internal Me_4Si as standard.

The Pyridinium Salts (5a—c).—Equimolar quantities of nicotinamide and the appropriate benzyl halide were heated in ethanol under reflux for 2 h. The precipitated salts were collected and crystallised from ethanol. 3-*Carbamoyl*-1-p-*nitrobenzylpyridinium bromide* (5a) (86%) had m.p. 249—250° (Found: C, 46·2; H, 3·6; N, 12·4. $C_{13}H_{12}BrN_3O_3$ requires C, 46·2; H, 3·6; N, 12·4%); 1-o-*bromobenzyl*-3-*carbamoylpyridinium bromide* (5b) (93%) had m.p. 231—232° (Found: C, 41·8; H, 3·2; N, 7·6. $C_{13}H_{12}Br_2N_2O$ requires C, 42·0; H, 3·25; N, 7·5%); 3-*carbamoyl*-1-p-*methylbenzylpyridinium chloride* (5c) (91%) had m.p. 258—260° (Found: C, 63·7; H, 5·7; N, 10·6. $C_{14}H_{15}ClN_2O$ requires C, 64·0; H, 5·75; N, 10·7%).

7-o-*Bromobenzyl*-3,4,4a,7-*tetrahydro*-3-*hydroxy*-3-*methyl*-2,7-*naphthyridin*-1(2H)-*one* (6; R = *o*-BrC_6H_4).—Triethylamine was added dropwise to a solution of 1-*o*-bromobenzyl-3-carbamoylpyridinium bromide (5b) (5 g) in water (100 ml) and acetone (10 ml) at 5—10° until pH 9—10 was reached. The mixture was stirred at 5—10° for 2 h and then allowed to warm to room temperature. The yellow precipitate was collected and triturated with ethanol to give light orange *crystals* (1·8 g, 37%), m.p. 169—170°; $\nu_{max.}$ 3300 (N–H), 1680, and 1580 cm^{-1} (NH–CO); δ [$(CD_3)_2SO$] 7·6 (2H, m, NH and OH), 6·9 (1H, s, 8-H), 6·0 (1H, d, 6-H), 4·5 (1H, q, 5-H), 3·6 (1H, m, 4a-H), 1·8 (2H, m, CH_2), 4·4 (2H, s, N·CH_2), and 1·3 (3H, s, CMe) (Found: C, 55·2; H, 5·0; N, 7·7. $C_{16}H_{17}BrN_2O_2$ requires C, 55·0; H, 4·9; N, 8·0%).

3,4,4a,7-*Tetrahydro*-3-*hydroxy*-3-*methyl*-7-p-*methylbenzyl*-2,7-*naphthyridin*-1(2H)-*one* (6; R = *p*-MeC_6H_4) was prepared similarly from (5c) (5·5 g) in water (100 ml) and acetone (10 ml). Trituration afforded a deep yellow powder (2·4 g, 42%), m.p. 150—151°; $\nu_{max.}$ 3300 (N–H), 1680, and 1580 cm^{-1} (NH–CO); δ [$(CD_3)_2SO$] 7·2 (1H, NH), 7·05 (1H, s, 8-H), 6·0 (1H, d, 6-H), 4·5 (1H, q, 5-H), 3·65 (1H, m, 4a-H), 2·0—1·5 (2H, m, CH_2), 4·3 (2H, s, N·CH_2), 1·4 (3H, s, MeC), and 2·3 (3H, s, ArMe) (Found: C, 72·0; H, 7·2; N, 9·8. $C_{17}H_{20}N_2O_2$ requires C, 71·8; H, 7·1; N, 9·85%).

4-*Substituted* 2H,7H-2,7-*Naphthyridine*-1,3-*diones* (7; R′ = CN or CO_2Et).—To a stirred solution of the salt (5a—c) (0·015 mol) in water (100 ml) was added at 5—10° either ethyl cyanoacetate (10 ml) or diethyl malonate (10 ml), followed dropwise by triethylamine until pH 9—10 was reached. After being stirred for 3 h the product, usually an oil, was collected and triturated with ethanol to induce crystallisation, and then recrystallised from a large volume of ethanol. Analytical and physical data are collected in Table 1 and spectroscopic data in Table 2.

3-*Ethoxycarbonylmethylene*-3,7-*dihydro*-2,7-*naphthyridin*-1(2H)-*ones* (8a—c).—These were obtained as for compounds (7) from salts (5a—c) (0·015 mol) in water (100 ml) and ethyl acetoacetate (10 ml). Recrystallisation was effected from ethanol. Analytical and physical data are collected in Table 3 and spectroscopic data in Table 4.

3-*Acetylmethylene*-3,7-*dihydro*-2,7-*naphthyridin*-1(2H)-*ones* (9a—c).—These were prepared as for (8) from the pyridinium salts (5a—c) and acetylacetone. Recrystallisation was performed from dimethylformamide. Analytical and physical data are collected in Table 3 and spectroscopic data in Table 4.

3-(α-*Ethoxycarbonylethylidene*)-3,7-*dihydro*-2,7-*naphthyridin*-1(2H)-*ones* (10b *and* c).—A solution of 1-*o*-bromobenzyl-3-carbamoylpyridinium bromide (5b) (10 g) in water (150 ml) was stirred and treated at 5—10° with ethyl α-methylacetoacetate (15 ml). Triethylamine was added dropwise until pH 9—10 was reached. After being stirred for 2 h the mixture was set aside at room temperature overnight. The brick-red *product* (10b) was collected and

TABLE 1

4-Substituted 2,7-naphthyridine-1,3-diones (7)

R	R′	Form (M.p.)	Molecular formula	% Yield	Found (%) C	H	N	Required (%) C	H	N
p-$O_2N{\cdot}C_6H_4$	CN	Deep red powder (>340°)	$C_{16}H_{10}N_4O_4$	23	59·4	3·2	17·2	59·6	3·1	17·4
o-BrC_6H_4	CN	Salmon pink powder (>330°)	$C_{16}H_{10}BrN_3O_2$	17	54·3	3·0	11·7	53·95	2·8	11·8
p-MeC_6H_4	CN	Grey powder (>355°)	$C_{17}H_{13}N_3O_2$	6	70·0	4·5	14·3	70·1	4·5	14·4
p-$O_2N{\cdot}C_6H_4$	CO_2Et	Yellow powder (292—294°)	$C_{18}H_{15}N_3O_6$	37	58·2	4·2	11·3	58·5	4·1	11·4
o-BrC_6H_4	CO_2Et	Bright yellow needles (254—255°)	$C_{18}H_{15}BrN_2O_4$	17	53·2	3·8	6·8	53·6	3·75	6·95
p-MeC_6H_4	CO_2Et	Yellow needles (229—230°)	$C_{19}H_{18}N_2O_4$	10	66·8	5·4	8·1	67·4	5·4	8·3

TABLE 2

Spectral data of 4-substituted 2,7-naphthyridine-1,3-diones (7)

R	R′	$\nu_{max.}$/cm^{-1}	NH	δ[$(CD_3)_2SO$] 8-H	6-H	5-H	ArH	N·CH_2	Others
p-$O_2N{\cdot}C_6H_4$	CN	3500 (NH), 2200 (CN), 1690 ($CONH_2$), 1530 (NO_2)	11·1(m)	8·8(s)	8·1(d)	7·06(d)	7·6—8·14	5·58(s)	
o-BrC_6H_4	CN	3400 (NH), 2200 (CN), 1690 (CO), 1630 (CO)	11·24(m)	8·8(s)	8·0(d)	7·2(d)	7·2—7·9	5·6(s)	
p-MeC_6H_4	CN	3400 (NH), 2200 (CN), 1690 (CO), 1630 (CO)		8·8(d)	8·0(d)	7·1(d)	7·2	5·4(s)	ArMe 2·3(s)
p-$O_2N{\cdot}C_6H_4$	CO_2Et	3450 (NH), 1690—1620 (CO), 1525 (NO_2)	10·8(m)	8·8(s)	8·2(d)	7·7(d)	8·4—7·5	5·4(s)	Et 4·1(q), 1·2(t)
o-BrC_6H_4	CO_2Et	3450 (NH), 1690 (CO)	10·63(m)	8·65(s)	7·9(m)	7·3(m)	7·3—7·9	5·4(s)	Et 4·19(q), 1·11(t)
p-MeC_6H_4	CO_2Et	3500(NH), 1590 (CO), 1610 (CO)	10·7(m)	8·7(s)	7·8(d)	7·7(d)	7·2	5·3(s)	Et 4·2(q), 1·2(t); ArMe 2·3(s)

recrystallised from ethanol; yield 0·8 g (7%), m.p. 225—226°; $\nu_{max.}$ 3400 (OH), 1680, and 1570 cm^{-1} (NH–CO); δ [$(CD_3)_2SO$] 8·3 (1H, s, 8-H), 7·1 (1H, d, 6-H), 6·6 (1H, d, 5-H), 5·1 (2H, s, N·CH_2), 5·6 (1H, s, 4-H), 4·1 (2H, q, CH_3·CH_2), 1·8 (3H, s, CH_3–C=C), and 1·2 (3H, t, CH_3·CH_2) (Found: C, 57·9; H, 4·7; Br, 19·3; N, 6·6. $C_{20}H_{19}BrN_2O_3$ requires C, 57·8; H, 4·6; Br, 19·2; N, 6·75%).

To a stirred solution of 3-carbamoyl-1-*p*-methylbenzylpyridinium chloride (5c) (5 g) in water (100 ml), ethyl α-methylacetoacetate (10 ml) was added at 5—10°, followed dropwise by triethylamine until the solution remained at pH 9—10. After being stirred for 4 h and set aside overnight the product (10c) was collected. Recrystallisation from dimethylformamide gave bright red *plates* (0·45 g, 6%), m.p. 213—214°; $\nu_{max.}$ 3400 (OH), 1685, and 1580 cm^{-1}; δ [$(CD_3)_2SO$] 12·3 (1H, m, NH), 8·2 (1H, s, 8-H), 7·2 (1H, d, 6-H), 6·6 (1H, d, 5-H), 5·05 (2H, s, N·CH_2), 5·6 (1H, s, 4-H), 4·1 (2H, q, CH_3·CH_2), 1·8 (3H, s, CH_3–C=), 2·3 (3H, s, ArMe), and 1·2 (3H, t, CH_3·CH_2) (Found: C, 71·9; H, 6·4; N, 7·8. $C_{21}H_{22}N_2O_3$ requires C, 72·0; H, 6·3; N, 8·0%).

3-Ethoxycarbonylmethylene-3,7-dihydro-4-methyl-2,7-naphthyridin-1(2H)-ones (11a—c).—The reactions were conducted as for (8) and the products were recrystallised from dimethylformamide. Compound (11a) was obtained from 3-carbamoyl-1-*o*-nitrobenzylpyridinium bromide (5a) as rust-coloured *prisms* (27%), m.p. 232—233°; $\nu_{max.}$ 1670 (CO_2Et), 1590 (CONH), and 1530 cm^{-1} (NO_2); δ [$(CD_3)_2SO$] 8·2 (1H, s, 8-H), 7·35 (1H, d, 6-H), 6·7 (1H, d, 5-H), 5·2 (2H, s, N·CH_2), 4·5 (1H, s, EtO_2C·C*H*), 4·0 (2H, q, CH_3·CH_2), 1·7 (3H, s, CH_3–C=), and 1·2 (3H, t, CH_3·CH_2) (Found: C, 62·7; H, 4·9; N, 10·9. $C_{20}H_{19}N_3O_5$ requires C, 63·0; H, 5·0; N, 11·0%). Compound (11b) was obtained from 1-*o*-bromobenzyl-3-carbamoylpyridinium bromide (5b) as scarlet *prisms* (21%), m.p. 226—227°; $\nu_{max.}$ 3400 (OH), 1675, and 1580 cm^{-1} (NHCO); δ [$(CD_3)_2SO$] 8·2 (1H, d, 8-H), 6·7 (1H, d, 5-H), 5·15 (2H, s, N·CH_2), 4·5 (1H, s, EtO_2C·C*H*), 4·0 (2H, q, CH_3·CH_2), 1·7 (3H, s, CH_3–C=), and 1·2 (3H, t, CH_3·CH_2) (Found: C, 57·5; H, 4·7; N, 6·8. $C_{20}H_{19}BrN_2O_3$ requires C, 57·8; H, 4·6; N, 6·75%). Compound (11c) was obtained from 3-carbamoyl-1-*p*-methylbenzyl chloride (5c) as bright red *plates* (20%), m.p. 208—209°; $\nu_{max.}$ 1670, 1580 (CONH), 1620, and 1150 cm^{-1} (CO_2Et); δ [$(CD_3)_2SO$] 8·2 (1H, d, 8-H), 7·3 (1H, d, 6-H), 6·7 (1H, d, 5-H), 5·05 (2H, s, N·CH_2), 4·5 (1H, s, EtO_2C·C*H*), 4·05 (2H, q, CH_3·CH_2), 1·7 (3H, s, CH_3–C=), 1·2 (3H, t, CH_3·CH_2), and 2·3 (3H, s, ArMe) (Found: C, 72·4; H, 6·3; N, 7·8. $C_{21}H_{22}N_2O_3$ requires C, 72·0; H, 6·3; N, 8·0%).

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TABLE 3

3-Substituted 2,7-naphthyridin-1(2*H*)-ones

Compound	Form (M.p.)	Molecular formula	% Yield	Found (%) C	Found (%) H	Found (%) N	Required (%) C	Required (%) H	Required (%) N
(8a)	Scarlet powder (218—220°)	$C_{19}H_{17}N_3O_5$	6	61·8	4·7	11·2	62·1	4·7	11·4
(8b)	Dark orange crystals (242—243°)	$C_{19}H_{17}BrN_2O_3$	5	56·8	4·3	6·7	56·9	4·3	7·0
(8c)	Orange plates (215—216°)	$C_{20}H_{20}N_3O_3$	20	71·5	5·9	8·1	71·4	6·0	8·3
(9a)	Deep red powder (255—256°)	$C_{18}H_{15}N_3O_4$	7	63·8	4·5	12·6	64·1	4·5	12·5
(9b)	Buff needles (268—269°)	$C_{18}H_{15}BrN_2O_2$	12	57·8	4·0	7·4	58·2	4·1	7·55
(9c)	Golden brown plates (221—222°)	$C_{19}H_{18}N_2O_2$	4	74·2	6·1	8·9	74·5	5·9	9·15

TABLE 4

Spectral data of 3-substituted 2,7-naphthyridin-1(2*H*)-ones

Compound	$\nu_{max.}$/cm^{-1}	NH	δ[$(CD_3)_2SO$] 8-H	ArH	6-H	5-H	CO·CH=	4-H	N·CH_2	Others
(8a)	1670, 1590, 1140		8·3(s)	8·3—7·5	7·2(d)	6·5(d)	4·43(s)	5·4(s)	5·2(s)	Et 4·0(q), 1·1(t)
(8b)	1680, 1580, 1140	11·4(m)	8·1(s)	7·3—7·8	7·2(d)	6·6(d)	4·65(s)	5·3(s)	5·5(s)	Et 4·2(q), 1·4(t)
(8c)	1680, 1580, 1140		8·2(s)	7·2(s)	7·35(m)	6·5(d)	4·48(s)	5·35(s)	5·0(s)	Et 4·0(q), 1·1(t); ArMe 2·3(s)
(9a)	1660, 1620, 1170	11·4(m)	8·5(s)	8·4—7·5	7·35(d)	6·8(d)	5·05(s)	5·45(s)	5·3(s)	Ac 1·9(s)
(9b)	1680, 1600, 1170		9·7(s) *	7·5(m)	8·7(d)	8·1(d)		6·9(s)	6·0(s)	Ac 2·3(s)
(9c)	1670, 1600, 1170		8·5(s)	7·2(s)	7·4(d)	6·8(d)	5·1(s)	5·45(s)	5·14(s)	Ac 1·9(s); ArMe 2·3(s)

* In $CF_3·CO_2H$.