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Thio and Nitrogen Analogues of Acronycine[1,2]

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The thio, thio acetyl, oxime, and several hydrazone and azine derivatives of the antitumor alkaloid acronycine (1) were prepared. NMR spectroscopy was used to study the configurations around the C=N double bond in these acronycine derivatives. In the hydrazones and azines of acronycine the N-N bond assumes a *syn* configuration to the C_6-OCH_3 group, while the N-O bond in the oxime and the N-N bond in noracronycine hydrazone and azines assumes an *anti* configuration.

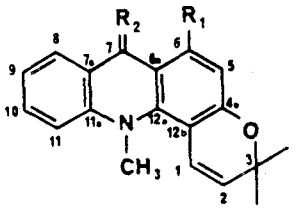
Key words : Acronycine, Thio analogues, Nitrogen analogues, Configuration assignments.

INTRODUCTION

Acronycine (1) remains one of the most broadly active antitumor alkaloids known (Svoboda *et al.*, 1966; Cordell and Suffness, 1987). However, its low solubility in aqueous systems is regarded as a substantial impediment to its' effective clinical evaluation (Hewitt, 1968). Complex formation with polyvinylpyrrolidone and other ligands (Svoboda *et al.*, 1971) and synthesis of prodrugs have been used to enhance water solubility. For example, the highly water soluble O-methyl fluorosulfonate derivative (2) has been prepared (Smithwick, 1974), but no details relating to its biological activity are available. The 7-anil (3) (Dimmock *et al.*, 1979) and the 7-acetyl (4) (Bourne *et al.*, 1979) derivatives have been described, but the half lives were too short under environmental conditions approximating those found *in vivo* (Repta *et al.*, 1977).

Hydrolysis of the 7-acetyl derivative to regenerate acronycine was shown to involve the fission of both acyl and aryl oxygen bonds, and thus the rate could not be altered by modifying the acyl function. Thermodynamic calculations suggested that the 7-thio analog (5) would have a longer half life (Repta *et al.*, 1977), and thioacronycine (6) (Smolders *et al.*, 1982) was prepared, but its acetyl derivative has not yet been synthesized. Here, we describe an improved synthesis of thioacronycine (6), conversion to its acetyl derivative, and the formation of several other water-soluble derivatives of varying stability.

The *syn-anti* isomerism of the oximes and hydrazones of both aliphatic and aromatic aldehydes and ketones, and the utilization of nmr techniques in the assignment of their configurations have been well documented (McCarthy, 1970). The hydrazone of 10-methyl-9(10H)acridone (7) has been reported (Schonberg and Sidky, 1959), but here *syn-anti* isomerism is a moot issue. We have used ^{1}H-nmr to assign the configuration of acronycine oximes hydrazones and azines.



	R_1	R_2
1	OCH_3	O
3	OCH_3	NC_6H_5
6	OCH_3	S
8	OH	S
12	OH	O
14	OCH_3	NNH_2
15	OCH_3	$NNHC_6H_3(NO_2)_2$
16	OCH_3	NOH
17	OCH_3	$NN(CH_3)_2$
18	OCH_3	$NNC(CH_3)_2$
19	OCH_3	$NNC(CH_3)CH_2CH(CH_3)_2$
20	OH	NNH_2
21	OH	$NNC(CH_3)_2$

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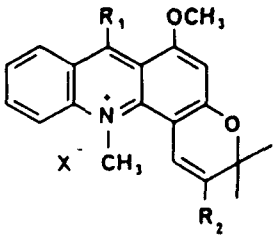
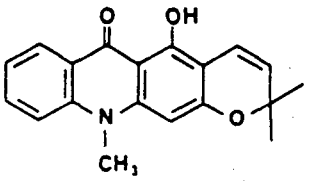


	R_1	R_2	X^-
2	OCH_3	H	FSO_3
4	$OCOCH_3$	H	ClO_4
5	$SCOCH_3$	H	ClO_4
9	Cl	H	PO_2Cl_2
10	SCH_3	H	I
11	SCH_3	$COCH_3$	ClO_4



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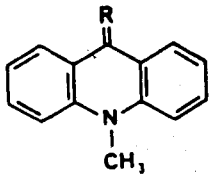

	R
7	$NNHH_2$
22	$NN(CH_3)_2$
23	O
24	NOH
25	S

MATERIAL AND METHODS

The melting points were recorded on a Kofler hot stage microscope and are uncorrected. IR spectra were recorded as KBr pellets with a Nicolet MX-I FT-IR interferometer; absorption bands are recorded in wave numbers (cm^{-1}). UV spectral data were measured in methanol with a Beckman DU-7 spectrometer. ^{1}H-NMR spectra and the nOe experiments were performed on a Varian XL-300 instrument and ^{13}C-nmr spectra were carried out on a Nicolet NMC 360 instrument operating at 90.8 MHz in deuterochloroform using tetramethylsilane as the internal standard. Mass spectra were recorded on a Varian MAT 112S double focussing spectrometer at 70 eV.

Satisfactory elemental analyses were obtained for all the new compounds except when this was not possible due to the lability or extreme hygroscopic nature of the compounds. In such cases the consistency of the ^{1}H, ^{13}C and mass spectra, and the formation of only a single spot on thin layer chromatography in several solvent systems was used as the criterion for purity. Microanalyses were carried out at the Microanalysis Laboratories, Roger Adams Laboratories, University of Illinois at Urbana-Champaign, Champaign, IL.

Chloroacronycinium dichlorophosphate (9).-To an ice-cold solution of acronycine (1, 1.0 g) in chloroform (10 ml), phosphorus oxychloride (3 ml) was added dropwise. The brown solid which separated was heated on a steam bath until the solid dissolved completely giving a magenta solution. Removal of excess phosphorus oxychloride *in vacuo at* 45°C produced a dark purple solid (9, 1.55 g); dec 180°C; UV (MeOH) λmax 258.5 (log ε 3.89), 308.5 (4.20), 369.5 (3.46), 385.5 (3.56) and 540 nm (3.12); IR νmax 1610, 1286, 1230, 1217, 1179, 1150, 1142, 1111, 759 and 668 cm^{-1}; ^{1}H-NMR ($CDCl_3$) d 8.72 (1H, dd, J = 8.3, 1.4, H-8), 8.27 (2H, m, H-10 and H-11), 7.84 (1H, ddd, J = 8.3, 7.2, 1.4, H-9), 6.79 (1H, d, J = 9.6, H-1), 6.70 (1H, s, H-5), 5.99 (1H, d, J = 9.6, H-2), 4.54 (3H, s, NCH_3), 4.14 (3H, s, OCH_3), and 1.66 (6H, s, 3-$(CH_3)_2$); ^{13}C-NMR ($CDCl_3$) δ 157.44 (s, C-4a), 155.03 (s, C-7), 155.01 (s, C-6), 144.53 (s, C-11a), 143.76 (s, C-12a), 142.36 (s, C-7a), 130.0 (d, C-10), 129.17 (d, C-8), 124.18 (d, C-9) 124.04 (d, C-2), 121.27 (d, C-1), 115.77 (d, C-11), 106.93 (s, C-6a), 104.72 (s, C-12b), 95.27 (d, C-5), 74.76 (s, C-3), 55.85 (q, CCH_3), 43.05 (q, NCH_3), and 26.75 (q, $CH_3 \times 2$); MS m/z (rel. int.) 340 (M^+, 2%), 293 (1), 274 (3), 273 (5), 261 (3), 255 (3), 243 (18), 242 (20), 159 (25) and 81 (100). The hygroscopic nature of the compound prevented the acquisition of analytical data.

Thioacronycine (6).-7-Chloroacronycinium salt (9, 1.55 g) (used without any further purification) was dissolved in ice-cold 10% aqueous sulfuric acid (300 ml) and added in a thin stream to a rapidly stirred, 20% aqueous solution of sodium thiosulfate at 5°C. The green precipitate was filtered, washed, dried and recrystallized from hot ethyl acetate to give green rods of 6 (0.98 g, 96%); mp 192°C (lit. (8) 186-187°C); UV (MeOH) λmax 295 (log e 2.59), 410 (2.12) and 459.5 (2.03); (+H^+) 264 (2.33), 3.05 (2.63), 391 (2.23), 471 (1.60) and 512 nm (1.54); IR νmax 1625, 1608, 1588, 1574, 1555, 1483, 1463, 1388, 1148 and 1139 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.65 (1H, dd, J = 8.2, 1.4, H-8), 7.59 (1H, ddd, J = 8.2, 7.2, 1.4, H-l0), 7.33 (1H, dd, J = 8.2, 1.4, H-11), 7.27 (1H, ddd, J = 8.2, 7.1, 0.5, H-9), 6.58 (1H, d, J = 9.6, H-1), 6.41 (1H, s, H-5), 5.54 (1H, d, J = 9.6, H-2), 3.95 (3H, s, NCH_3), 3.84 (3H, s,

OCH_3), 1.57 (3H, s, 3-CH_3) and 1.55 (3H, s, 3CH_3); ^{13}C-NMR ($CDCl_3$) δ 201.75 (s, C-7), 162.37 (s, C-4a), 158.79 (s, C-6), 141.37 (s, C-11a), 138.38 (s, C-12a), 136.10 (s, C-7a), 131.21 (d, C-10), 129.79 (d, C-8), 123.16 (d, C-9), 122.67 (d, C-2), 122.60 (s, C-6a), 121.36 (d, C-1), 115.29 (d, C-11), 101.99 (s, C-12b), 95.58 (d, C-5), 76.90 (s, C-3), 55.11 (q, OCH_3), 44.51 (q, NCH_3), and 26.59 (q, $CH_3 \times 2$); MS m/z (rel. int.) 388 (M^++I, 28%), 337 (M^+, 100), 336 (71), 322 (16), 306 (19), 305 (24), 204 (87), 292 (24), 274 (17), 161 (21), 160 (24), 146 (17) and 144 (14). *Anal.* Calcd. for $C_{20}H_{19}NO_2S$: C, 71.21; H, 5.64; N, 4.15; S, 9.5. Found: C, 71.46; H, 5.70; N, 4.18; S, 9.58. For small scale preparations the aqueous phase may be extracted with ethyl acetate to improve the yields.

Thioacetylacronycinium perchlorate (5). -Thioacronycine (6, 0.58 g), perchloric acid (5 drops) and methylene chloride (5 ml) were warmed on a water bath (40°C) until a purple solution was obtained, cooled in ice and acetic anhydride (5 drops) was added. The mixture was stirred at room temperature under anhydrous conditions for 2 hours and the solvents were removed *in vacuo* below 35°C and the residue dried over potassium hydroxide pellets in vacuo. The dark purple solid (68 mg) was shown to contain 80% w/w of thioacetylacronycinium perchlorate (5) by ^{1}H-NMR analysis; UV (MeOH) λmax 295.5 (log ε 4.25), 388 (3.77), 4.08 (3.41) and 525 nm (3.21); IR νmax 1710, 1618, 1606, 1580, 1572, 1479, 1460, 1141, 1089 and 637 cm^{-1}; ^{1}H-NMR ($CDCl_3$) d 8.43 (1H, dd, J = 8.0, 1.8, H-8), 8.12 (1H, m, H-10), 7.73 (2H, m, H-9 and H-11), 6.73 (1H, d, J = 9.7, H-1), 6.67 (1H, s, H-5), 5.74 (1H, d, J = 9.7, H-2), 4.35 (3H, d, NCH_3), 4.15 (3H, s, OCH_3) and 1.74 (6H, s, 3-$(CH_3)_2$); MS m/z (rel. int.) 380 (M^+, 2%), 338 (4), 330 (12), 318 (17), 292 (22), 280 (20), 268 (18), 180 (13), 130 (15), 68 (100) and 42 (72).

Thionoracronycine (8).-Thioacronycine (6, 0.056 g, 0.17 mmol) was dissolved in 10% methanolic hydrochloric acid (9 ml) and the solution evaporated to dryness under a stream of nitrogen. The black residue was pyrolysed in a Kugelrohr apparatus in vacuo at 180°C for 15 minutes and the deep red residue purified by preparative thin layer chromatography to give thionoracronycine (8, 0.021 g, 39%): maroon needles were obtained from methanol, mp 221°C (lit. (8) 223°C); UV (MeOH) λmax 490 (log ε 3.74) and 410 nm (4.26); IR νmax 2270, 1630, 1595 cm^{-1}; ^{1}H-NMR d, see Table I; ^{13}C-NMR ($CDCl_3$) δ 194.22 (s, C-7), 165.53 (s, C-6), 162.25 (s, C-4a), 144.77 (s, C-11a), 140.22 (s, C-12a), 134.25 (d, C-10), 130.74 (d, C-8), 130.34 (s, C-7a), 123.95 (d, C-1), 123.67 (d, C-2), 122.12 (d, C-9), 119.42 (s, C-6a), 116.63 (d, C-11), 101.47 (s, C-12b), 100.38 (d, C-5), 77.60 (s, C-3), 45.62 (q, NCH_3) and 27.60 (q, $CH_3 \times 2$); MS m/z (rel. int.) 323 (45%, M^+), 309 (19), 308 (100), 293 (35), 204 (22), 154 (25), 153 (20), 140 (16), 139 (15), 69 (39). *Anal* Calcd. for $C_{19}H_{17}NO_2S$: C, 70.59; H, 5.26; N, 4.33; S, 9.91. Found: C, 70.38; H, 5.37; N, 4.38; S, 9.87.

General Procedure for the Conversion of a Thioanalogue to the Hydrazone or Oxime.-To a stirred solution of thioacronycine (or thioacridone) (0.3 mmol) in 95% ethanol at 60°C the appropriate hydrazone was added (hydrazine hydrate for 7 and 14, 2,4-dinitrophenyl hydrazine for 15, *N.N*-dimethyl hydrazine for 17 and 22, and hydroxylamine for 16) (0.5 mmol), and heating continued until the green color of the solution disappeared or no thio derivative could be detected by thin layer chromatography. The solvent was removed *in vacuo,* and the residue was redissolved in chloroform, washed successively with 10% aqueous ammonium chloride, water, dried and evaporated. The N-substituted hydrazones and the azines were recrystallized from acetone-water.

Acronycine hydrazone (14).-The yellow solid (yield 100%) gave a single spot on silica gel tlc R_f 0.55 (toluene:ethyl acetate, 1:1), which appeared brown under uv light and turned red on exposure to iodine vapor; mp 138-140°C with dec; UV (MeOH) λmax 256 (log ε 3.99), 276.5 (4.03), 290 sh (2.96), 307 sh (3.83), and 395.5 nm (3.45); (+H$^+$) 284 (4.35), 320 (3.74), 364 (3.74) and 426.5 nm (3.58); IR νmax 3800, 1597, 1575, 1568, 1399, 1220, 1204, 1184, 1166, 1132 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, see Table I; ^{13}C-NMR ($CDCl_3$) δ 159.96 (s, C-6), 157.88 (s, C-4a), 149.79 (s, C-12a), 144.95 (s, C-11a), 143.52 (s, C-7), 130.01 (d, C-8), 129.87 (d, C-10), 124.57 (d, C-2), 124.19 (d, C-9), 122.28 (d, C-1), 121.68 (s, C-7a), 115.77 (d, C-11), 106.33 (s, C-6a), 104.16 (s, C-12b); 95.35 (d, C-5), 75.79 (s, C-3), 57.85 (q, OCH_3), 43.84 (q, NCH_3), and 27.01 (q, $CH_3 \times 2$); MS m/z (rel. int.) 336 (M^++H, 22%), 335 (M^+, 100), 320 (21), 319 (47), 304 (32), 288 (30), 152 (30) and 144 (30). *Anal.* Calcd. for $C_{20}H_{22}N_3O_4Cl$: C, 64.6; H, 5.92; N, 11.31; Cl, 9.55. Found: C, 64.4; H, 5.22; N, 11.25, Cl, 9.60.

Acronycine 2,4-Dinitrophenyl hydrazone (15).-Dark purple needles were obtained from ethanol (yield 83%); mp 252°C; UV (MeOH:$CHCl_3$, 9:1) λmax 228.5 (log ε 4.64), 263 (4.28), 275 (4.16), 308 (4.19) and 437 nm (4.31); (+H$^+$) 263.5 (4.28), 2.95 (4.52), 3.82 (4.28) and 444 nm (3.95); IR νmax 3490, 1614, 1605, 1586, 1567, 1515, 1501, 1385, 1312, 1205 and 1133 cm^{-1}; ^{1}H-NMR δ, see Table I; ^{13}C-NMR ($CDCl_3$) δ 159.08 (s, C-6), 157.68 (s, C-4a), 157.0 (s, C-1'), 149.38 (s, C-4'), 145.48 (s, C-11a), 144.57 (s, C-7), 143.80 (s, C-12a), 134.43 (s, C-2'), 130.33 (d, C-10), 130.29 (d, C-8), 129.12 (d, C-5'), 125.59 (d, C-2), 123.41 (d, C-9), 123.23 (d, C-3'), 122.21 (d, C-1), 121.46 (s, C-7a), 116.43 (d, C-11), 115.30 (d, C-6'), 106.01 (s, C-6a), 105.34 (s, C-12b), 95.53 (d, C-5), 76.94 (s, C-3), 57.12

Table I. Proton NMR Assignments of acronycine and Acridone Derivatives.[a]

	Proton													
Compound	1	2	5	8	9	10	11	OCH_3	NCH_3	$(CH_3)_2$	Other singals			
Actonycine (1)	6.53	5.50	6.31	8.39	7.24	7.61	7.34	3.98	3.83	1.55				
noractonycine (12)	6.56	5.51	6.28	8.40	7.33	7.74	7.44	-	3.92	1.53				
Thioacronycine (6)	6.58	5.54	6.41	8.65	7.27	7.59	7.33	3.95	3.84	1.56				
Thionoractonycne (8)	6.50	5.51	6.41	8.92	7.30	7.74	7.65	-	3.97	1.62				
Acronycine Hydrazone (4)	6.59	5.53	6.23	7.66	7.06	7.28	7.08	3.92	3.61	1.45				
Noracronycine Hydrazone (20)	6.59	5.53	6.28	8.41	7.33	7.74	7.47	-	3.93	1.51				
Acronycine Acetone Azine (18)	6.62	5.51	6.21	7.98	7.13	7.39	7.18	3.70	3.66	1.48	2.14	2.09		
Noracronycine Acetone Azine (21)	6.61	5.50	6.20	8.29	7.14	7.40	7.18	-	3.92	1.50	2.19	2.10		
Acronycine 4Me-2-Pentanone Azine (19)	6.58	5.50	6.17	7.94	7.15	7.38	7.14	3.67	3.57	1.54	2.08	2.20	2.10	0.98
Acronycin N, N-Dimethyl Hydrazone (17)	6.58	5.48	6.19	7.82	7.04	7.22	7.05	3.87	3.64	1.49	2.66			
Acronycine 2, 4 1)np Hydrazone (15)	6.61	5.58	6.43	7.96	7.18	7.47	7.20	3.98	3.71	1.50	9.12	8.25	8.6	NH9.3
Acronycine Oxime (16)	6.50	5.50	6.28	8.68	7.08	7.79	7.18	3.89	3.61	1.48				
			1	1/8	2/7	3/6	4/5		NCH_3					
N-Methyl Acridone (23)				8.57	7.27	7.71	7.55		3.89					
N-Methyl Thioacridone (25)				9.20	7.36	7.78	7.61		3.99					
Acridone N, N-Dimethyl Hydrazone (22)			9.08	8.20	7.02	7.38	7.10		3.51					
Acridone Oxime (24)			8.83	7.90	7.09	7.42	7.05		3.53					

[a]Data obtained in $CDCl_3$ with TMS ($\delta_{TMS}=O_{ppm}$) as internal standard.

(q, OCH_3), 44.62 (q, NCH_3) and 27.84 (q, $CH_3 \times 2$); MS m/z (rel. int.) 502 (M^++H, 20%), 501 (M^+, 67), 484 (12), 425 (27), 306 (31), 217 (16), 191 (14) and 167 (13). Anal. Calcd. for $C_{26}H_{23}N_5O_6$: C, 62.28; H, 4.59; N, 13. 95. Found: C, 62.32; H, 4.61; N, 14.01.

Acronycine oxime (16).-Yellow needles were obtained from MeOH-$CHCl_3$ (yield 75%); mp 238°C; UV (MeOH) λmax 275 (log ε 4.20), 290 (4.12), 361 (3.52) and 402 nm (3.23); (+H^+) 282.5 (4.41), 320 (3.79), 362.5 (4.0) and 423 nm (3.56); IR νmax 3400, 1601, 1572, 1567, 1439, 1393, 1138, 1130, 1093, 981 and 765 cm^{-1}; ^{1}H-NMR δ, see Table 1; ^{13}C-NMR ($CDCl_3$) δ 158.22 (s, C-6), 156.38 (s, C-4a), 145.16 (s, C-11a), 143.63 (s, C-7), 143.06 (s, C-12a), 130.64 (d, C-10), 130.06 (d, C-8), 124.66 (d, C-2), 120.41 (d, C-9), 120. 70 (d, C-1), 120.04 (s, C-7a), 116.52 (d, C-11), 107.02 (s, C-6a), 104.58 (s, C-12b), 95.06 (d, C-5), 76.05 (s, C-3), 56.34 (q, OCH_3), 44.20 (q, NCH_3), and 27.29 (q, $CH_3 \times 2$); MS m/z (rel. int.) 337 (M^++H, 12%), 336 (M^+, 54), 322 (22), 321 (100), 319 (42), 306 (33), 289 (31), 262 (10), 152 (21) and 102 (15). *Anal.* Calcd. for $C_{20}H_{20}N_2O_3$: C, 71.42; H, 5.95; N, 8.33. Found: C, 70.68; H, 5.84; N, 8.15.

Acronycine *N,N*-dimethyl hydrazone (17).-7-Chloroacronycinium dichloro phosphate salt prepared as described above from acronycine (1, 1.25 g, 3.9 mmol) was dissolved in tetrahydrofuran: 10% aqueous sulfuric acid (50 ml, 9:1), 1,1-dimethyl hydrazone (0.5 ml, 6.6 mmol) was added and the mixture warmed at 45°C until the solution turned orange. The reaction mixture was evaporated to dryness, washed with 10% aqueous ammonium chloride, dried, evaporated and the residue recrystallized from acetone-water to afford pale yellow rods of 17 (0.993 g, 70.4%); mp 168°C; UV (MeOH) λmax 284.5 (log ε 4.65), 368 (4.08) and 436 nm (3.55); IR νmax 1604, 1594, 1573, 1565, 1393, 1208, 1197, 1126, 1093 and 748 cm^{-1}; ^{1}H-NMR δ, see Table 1; ^{13}C-NMR ($CDCl_3$) δ 158.99 (s, C-6), 157. 26 (s, C-4a), 144.47 (s, C-7), 144.43 (s, C-11a), 144.40 (s, C12a), 131.88 (d, C-8), 129.71 (d, C-10), 124.44 (d, C-2), 123.88 (d, C-9), 122.93 (d, C-l), 117.03 (d, C-11), 108.81 (s, C-6a), 104.66 (s, C-12b), 96.46 (d, C-5), 75. 77 (s, C-3), 56.05 (q, OCH_3), 43.53 (q, NCH_3), and 27. 24 (q, $CH_3 \times 2$); MS m/z (rel. int.) 364 (M^++H, 25%), 363 (M^+, 100), 349 (17), 348 (65), 309 (28), 305 (25), 290 (35), 276 (26), 275 (31) and 166 (14). *Anal.* Calcd. for $C_{22}H_{25}N_3O_2$: C, 72.72; H, 6.89; N, 11.57. Found: C, 72.77; H, 6.98; N, 11.47.

Acronycine acetone azine (18).-A solution of acronycine hydrazone (0.08 g) in acetone (50 ml) was allowed to evaporate at room temperature in a petri dish. The residue was purified by preparative thin layer chromatography, and recrystallized from acetone-water to afford 18 (0.056 g, 63%) as dark brown rhomboids, mp 167°C; UV (MeOH) λmax 230 (log ε 4.23), 300 (4.14) and 380 nm (4.01), (+H^+) 225 (4.25), 310 (4.15), 395 (4.05) and 448 (3.97); IR νmax 1625, 1600, 1587, 1495, 1470, 1430, 1408, 1230 and 1150 cm^{-1}; ^{1}H-NMR δ, see Table I; MS m/z (rel.int.) 376 (M^++H, 7%), 375 (M^+, 25), 346 (7), 345 (26), 344 (100), 319 (14), 314 (12), 304 (12), 86 (26), 84 (31), and 69 (58). *Anal.* Calcd. for $C_{23}H_{25N3}O_2$: C, 73.60; H, 6.66; N, 11.2. Found: C, 73.56; H, 6.78; N, 10.96.

Acronycine 4-methyl-2-pentanone azine (19).-On heating a solution of acronycine hydrazone (0.097 g) in commercially available absolute ethanol (Aldrich R) containing 4-methyl-2-pentanone as the stabilizer, a more polar compound was formed. Purification by preparative thin layer chromatography followed by recrystallization from acetone-water gave orange nee-

dles of 19 (0.036 g, 36%), mp 187°C; UV (MeOH) λmax 230 (log ε 4.60), 305 (3.80) and 280 nm (3.25), (+H^+) 230 (4.10) and 508 nm (3.50); IR νmax 1626, 1598, 1578, 1462, 1393, 1205, 1134, 755 and 650 cm^{-1}; ^{1}H-NMR δ, see Table 1; MS m/z (rel. int.) 417 (M^+, 22%), 403 (33), 402 (99), 387 (25), 386 (76), 377 (39), 376 (100), 361 (36), 345 (63), 344 (43), 321 (29), 319 (40), 306 (37), 304 (49), 291 (55), 275 (49), 144 (28) and 137 (26). *Anal.* Calcd. for $C_{26}H_{31}N_3O_2$: C, 74.8; H, 7.4; N, 10.1. Found: C, 74.2, H, 7.34: N, 10.0.

Noracronycine hydrazone (20).-Orange red needles were obtained from ethanol, mp 189°C; UV (MeOH) λmax 225 (log ε 3.95), 257 (3.85) and 405 (3.05), (+H^+) 225 (4.10) 280 (4.00), 390 (3.51) and 510 nm (3.35); IR νmax 3019, 1630, 1596, 1215, 1137, 669, and 618 cm^{-1}; ^{1}H-NMR δ see Table I; MS m/z (rel. int.) 322 (M^++H, 7%), 321 (M^+, 30), 305 (92), 304 (11), 292 (100), 289 (62), 277 (18), 146 (27), and 77 (40). Anal. Calcd. for $C_{19}H_{19}N_3O_2$: C, 71.0; H, 5.9; N, 13.1. Found: C, 71.2; H, 5.8; N, 13.2.

Noracronycine acetone azine (21).-On standing a solution of noracronycine hydrazone in acetone for 2-3 days at room temperature or on heating under reflux for 30 minutes a more polar compound was formed. Purification by preparative thin layer chromatography followed by crystallization from acetone gave brown needles of 21, mp 188-190°C; UV (MeOH) λmax 254 sh (log ε 4.1), 283.5 (4.32), 295 (4.25), 310 (4.02) and 299 nm (3.45); IR νmax 3300, 1691, 1611, 1608, 1589, 1571, 1567, 1548, 1536, 1332 and 1145 cm^{-1}; ^{1}H-NMR δ, see Table 1; MS m/z (rel. int.) 362 (M^++1, 26%), 361 (M^+, 60), 345 (31), 345 (100), 330 (18), 292 (20), 291 (16), 165 (18), and 98 (24).

10-Methyl-9-acridathione (25).-10-Methyl-9(10-H) acridone (1 g, 4.7 ml) was dissolved in chloroform (53 ml) and phosphorus oxychloride (1 ml) was added. The mixture was heated at 60°C for 15 minutes. Following removal of the solvents *in vacuo* the residue was dissolved in tetrahydrofuran: 10% aqueous sulfuric acid (9:1, 100 ml) and an aqueous solution of sodium thiosulfate was added with rapid stirring. The orange precipitate was filtered and recrystallized from methanol as fine red needles, mp 270°C (lit. (8) 267°C); UV (MeOH) λmax 241 (log ε 3.96), 288 (3.90), 458.5 (3.91) and 484 nm (3.93); IR νmax 1609, 1575, 1546, 1499, 1443, 1225, 1181, 1059, 991 and 746 cm^{-1}; ^{1}H-NMR δ, see Table 1; MS m/z (rel. int.) 226 (M^++ 1, 80%), 225 (M^+, 100), 224 (71), 210 (87), 209 (73), 181 (38), 180 (26), 166 (84), 112 (99), 111 (33), and 105 (23). *Anal* Calcd. for $C_{14}H_{11}NS$: C, 74.66; H, 4.89; N, 6.22; S, 14.22. Found: C, 74.45; H, 4.81; N, 6.13; S, 14.32.

N,N-Dimethyl Hydrazone of 9-Acridone (22).-Yellow needles were obtained from acetone, mp 117°C; UV (MeOH) λmax 243.5 (log ε 4.02), 285.5 sh (3.69), and 399 nm (3.74); IR νmax 1596, 1591, 1482, 1467, 1453, 1360, 1208, 1173, 980 and 750 cm^{-1}; ^{1}H-NMR δ, see Table I; MS m/z (rel. int.) 252 (M^++ 1, 13%), 251 (M^+, 83), 237 (16), 236 (100), 221 (8), 208 (6), 194 (15), 193 (53), 192 (22), 191 (7), 178 (5), 165 (5), 125 (11), and 77 (5). Calcd. for $C_{16}H_{17}N_3$: C, 76.49; H, 6.77; N, 16.73. Found: C, 76.52; H, 6.92; N, 16.82.

Hydrolysis of Acronycine Derivatives.-A weighed sample (approx. 5 mg) of the derivative was dissolved in water (200 ml) and equilibrated at 60°C (at room temperature the rates of hydrolysis were found to be too slow for convenient measurement) in an enclosed system fitted with a reflux condenser and a diaphragm. Aliquots were withdrawn with the aid of a syringe at regular intervals and the concentration of the starting derivative was estimated by measuring the uv absorption at a wavelength characteristic of the compound. Linear correlations were established for time vs. log concentration curves by the least squares method. Correlation values (r) of 0.95 were observed for the curves.

RESULTS AND DISCUSSION

Early attempts to synthesize thioacronycine (6) directly from 1 afforded thionoracronycine (8) (Repta *et al.,* 1977), although in hexamethylphosphorus triamide, 6 was obtained in 45% yield (Smolders *et al.*, 1982). Thio analogs of acridones (Hunig and Hermann, 1960; Gagan, 1973) have also been prepared from the corresponding cholro compound with aqueous sulfide ion in satisfactory yields.

Acronycine, when treated with phosphorus oxychloride at room temperature, formed a brown solid which yielded a magenta solution when warmed briefly at 45°C. Removal of excess phosphorus oxychloride *in vacuo* left a dark purple solid, soluble in water and in polar organic solvents, including chloroform. This purple solid, the 7-chloroacronycinium salt 9, could not obtained crystalline and did not give a satisfactory elemental analysis due to its instability and extreme hygroscopic nature. On exposure to air, or in solution, the salt decomposed quantitatively to acronycine (1). The structure of 9 was supported by the UV absorption maximum of 540 nm, a M^+ion at *m/z* 341, and its ^{1}H and ^{13}C nmr spectra. No vinylogous amide band was observed at 1624 cm^{-1} in the ir spectrum, and the ^{13}C nmr signal for the carbonyl function was absent.

Treatment of an aqueous solution of 9 with aqueous sodium thiosulfate at 10°C produced thioacronycine (6) as a green precipitate in quantitative yield which could by recrystallized as green shiny rods from ethyl acetate, or fine brown needles from chloroform or methanol. In contrast to 9-acridanones, the

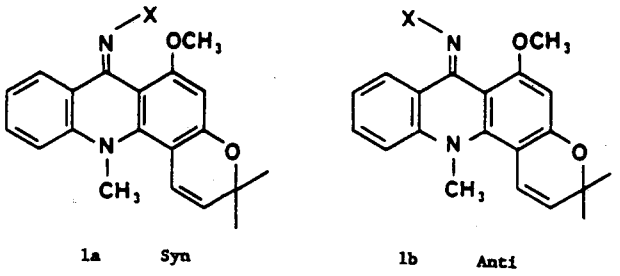

Fig. 1.

corresponding thiones are reported to be readily converted to thioesters with acetic anhydride, acetyl chloride and *p*-toluene sulfonyl chloride in the presence of alkali (Hunig and Hermann, 1960). In our hands, none of the above reagents was satisfactory, since all reacted only at high temperature where thioacronycine underwent hydrolysis to 1. Iodomethane afforded the soluble thio ether 10 in excellent yield, but this underwent hydrolysis only in warm alkali.

Thioacetyl acronycinium salt (5) was prepared by low temperature treatment of 6 with aceic anhydride. Conversion was estimated by monitoring the three-proton singlet at 2.45 ppm of the thioacetyl function. At high temperatures, acylation occurred predominantly to afford 2-acetylthioacronycine (11), as indicated by a one-proton singlet for H-1 at 7.72 ppm and the downfield shift of the geminal dimethyl signal to 1.9 ppm. Acronycine itself does not undergo acylation at the 2-position under similar conditions.

On exposure to a variety of bases, the salt 5 decomposed to give 6, and hydrolysis in traces of acid yielded 1. Consequently, the hydrolysis mechanism under *in vivo* conditions should play an important role in determining the utility of this salt as a prodrug since the release of the highly insoluble 6 within the system would be undesirable. As anticipated, this salt had an increased water solubility and a longer half life (18.5 hrs at 60°C) in neutral media compared with acetyl acronycinium salt (25 minutes at 25°C) (Fig. 1).

Thionoracronycine (8) could not be prepared from noracronycine (12) by this method since the latter compound undergoes dimerization and rearrangement to isonoracronycine (13) (Funayama *et al.*, 1984). Pyrolysis of the hydrochloride salt of thioacronycinium *in vacuo* gave 8 in moderate yield.

Thioacronycine (6) reacts readily with amines, hydrazines and hydroxylamines to give anils, hydrazones and oximes, respectively, on warming an alcoholic solution of 6 and the appropriate reagent. Hydrazone 14, a hygroscopic solid, undergoes further reaction on exposure to air and must be stored under nitrogen. The products of this reaction will be discussed in a subsequent paper. It also reacts quite readily with aliphatic ketones at room temperature to give the corresponding azines. The 2,4-dinitro phenyl hydrazone 15 and the oxime 16 are stable compounds. The anils were not further investigated as they are reported to be rather unstable (Bourne *et al.* 1979). The N-substituted hydrazones of acronycine could be prepared by reaction of 9 with the appropriate hydrazine, but the oxime could not be prepared by this route.

Although potentially both *syn* and *anti* isomers of the hydrazones and oximes could be produced, in all preparations only a single isomer was formed, and it was considered that the isomer in which the N-N bond is *anti* to the methoxy function for steric reasons, was the thermodynamically favored product. Even though the proton nmr spectra of the nitrogen analogs were found to be very similar to that of acronycine (Funayama *et al.*, 1984), some variation in the chemical shifts of the ring A protons, particularly H-8 which is located *peri* to the C=N function, was observed (Table I). In the hydrazones (14, 15 and 17) and azines (18 and 19), H-8 is considerably shielded compared to that of acronycine, while in the oxime 16, the hydrazone 20 and the azine 21 of noracronycine, the same H appeared to be deshielded.

NMR spectroscopy, in addition to dipole moment measurement, has been the most effective tool in assigning the configuration around the C=N bond in hydrazones, oximes and azines (McCarthy, 1970; Martin and Martin, 1972). The shielding and deshielding effects around the C=N bond vary depending on the distance and environment of the protons under consideration. In the case of the aldoximes or their ethers, the bay region of the C=N-O plane (i.e. the side of the N-O bond) is shown to be deshielded compared to the opposite side. Thus the aldoxime proton appears at a higher field when *anti* to the N-O bond and at a lower field when it is *syn*. The α-protons both in aldoximes and in ketoximes, which are one more bond removed from the C=N bond, experience the reverse effect: the *syn* α-protons appear at higher field compared to the α-protons *anti* to the N-O bond in aliphatic solvents. Similarly, the aldehyde protons are deshielded when *syn* to the N-N bond compared to the protons in the *anti* configuration. The β-protons, on the other hand, appear at higher field when situated *anti* rather than a *syn* configuration. In aldazenes and ketazenes the β-protons which are *syn* to the N-N bond appear to be shielded compared with the protons oriented *anti*. The effect of the C=N bond on the chemical shifts of aromatic protons has not attracted attention.

In order to investigate the effect of the C=N bond on the acridone system, a model compound, the N,N-dimethyl hydrazone of 10-methyl-9(10H)-acridone, 22, was synthesized. The *peri* protons at C-1 and C-8 in

acridone 23 appeared at 8.57 ppm, while in 22, H-1 and H-8 appeared at 9.08 and 8.20 ppm. A significant NOE effect (12%) between the *N,N*-dimethyl protons and the proton appearing at 9.08 ppm permitted the unambiguous assignment of the latter signal to the proton *syn* to the N-N bond. In deuterochloroform the signal due to the *syn* proton appeared as a sharp doublet, while the signal due to the *anti* proton showed significant line broadening. Similarly, in the oxime of acridone, 24, the two *peri* protons appeared at 8.83 and 7.90 ppm, and the oxime hydroxy proton signal at 11.35 ppm showed a strong NOE effect (28%) with the more downfield signal. Therefore it is evident that in the hydrazones and oximes of acridone the *peri* proton *syn* to the N-X bond is deshielded ($\Delta\delta$ 0.37 ppm), while the corresponding *anti* proton is shielded ($\Delta\delta$ 0.51 ppm) compared to the acridone system. In 17, the N,N-dimethyl hydrazone of 1, H-8 (7.82 ppm) showed an upfield shift of 0.57 ppm (*anti* configuration). However, H-8 in the oxime 16 showed a downfield shift of 0.29 ppm compared to H-8 in 1, and must be *syn* to the N-O bond. Also, in the hydrazone of noracronycine, H-8 is deshielded compared to H-8 in acronycine, and therefore must have the same configuration as the oximes, i.e. H-8 is *syn* to the N-N bond. From this point we designate the structure in which the N-X bond projects towards the C_6-OMe as the *syn* isomer; when the same group points away from the OMe group it is designated as the *anti* isomer (Fig. 1).

Considering only steric factors, the *anti* isomer should be preferred over the *syn* isomer for both acronycine and noracronycine derivatives. However, for some, as yet unknown reason, the *syn* isomer is the favored configuration for the acronycine hydrazones and azines. Most hydrazones and oximes are known to exist as mixtures of *syn* and *anti* isomers even at room temperature. But when the acronycine derivatives were heated in the nmr probe up to 120°C, no significant change in the chemical shifts of either ^1H or ^{13}C signals were observed, thereby indicating the greater thermal stability of the individual isomers formed. Likewise, no change was observed when the solutions were exposed to uv irradiation. The *anti* configuration for the oximes and the noracronycine derivatives may be stabilized by the presence of hydrogen bonding between the C_6-OH proton and N-1. But the preference of the *anti* configuration for the oxime 16 cannot be explained on this basis. It has been shown previously that the dipole alignments in the azines of esters play an important role in determining the configuration (Keus and Warkentin, 1984), and similar forces may also be in operation in these derivatives.

The chemical changes at position 7 also affect the chemical shift of the NMe protons: in the hydrazone and oximes the NMe protons appear to be considerably shielded compared with their oxygen analogs, while de-*O*-methylation of acronycine has a pronounced shielding effect on the same protons due to the localization of the electrons on the carbonyl function by hydrogen bonding.

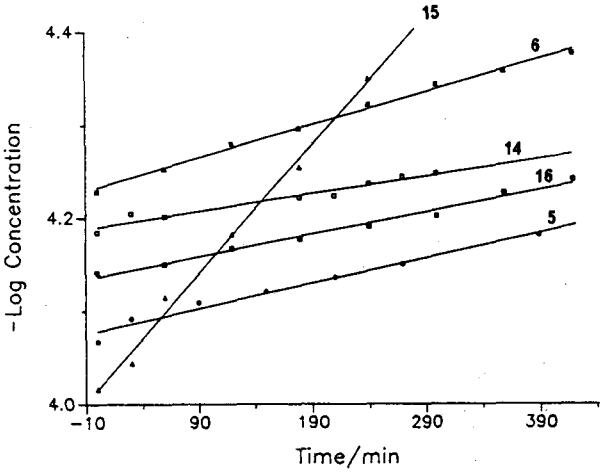

Fig. 2. Rate of Hydrolysis of Acronycine Derivatives in H_2O at 60°C

○ : Thioacetyl acronycinium salt (5) $t_{1/2}$	52±2 Hrs
● : Thioacronycine(6)	40±1
□ : Acronycine hydrazone (14)	75±6
▲ : Acronycine 2,4-DNP hydrazone (15)	10.8±0.4
■ : Acronycine oxime (16)	60±3

Stability of the derivatives: It has been shown that a minimum solubility of 30 mg/100 ml of water is required for the intravenous administration of the required dose of acronycine. The solubility of the oxime and hydrazone salts and the thioacronycinium salt far exceed this required minimum making them potentially suitable as prodrugs. However, since a prodrug should also have a certain stability, the hydrolytic rates of some of the representative compounds were measured. Because the rate of hydrolysis at room temperature was too slow for measurement, aqueous solutions were incubated in sealed vessels at 60°C and the concentration of the individual entities monitored by recording their uv absorbtion. The concentration versus time curves are shown in Fig. 2 and by comparison of the rates of hydrolysis of the hydrazone and the 2,4-dinitrophenylhydrazone it appears that the rate is dependent on the nature of the substituents on N-2, and thus may provide a way of modifying the hydrolytic rate as required.

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