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N-Quaternary Compounds

Part II.[1] The Synthesis of Dihydrothiazolo[3,2-a]pyridinium-8-oxides

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The syntheses of 8-hydroxy-5-methyldihydrothiazolo[3,2-a]pyridinium-3-carboxylate and analogues are described. The former substance was shown to be the racemic modification of a blue fluorescent substance isolated from bovine liver hydrolysates.

The blue fluorescent material isolated from bovine liver hydrolysates by Laland *et al.*[2] has been shown by chemical degradation work to be 8-hydroxy-5-methyldihydrothiazolo[3,2-a]pyridinium-3-carboxylate (I).[1] We now offer a synthetic confirmation of this structure.

The simplest synthetic approach to the dihydrothiazolo[3,2-a]pyridinium-8-oxide ring system appeared to be the addition of 1,2-disubstituted ethane derivatives to 3-hydroxypyrid-2-thione derivatives to form the 5-membered ring. In a similar way the recently described unsubstituted dihydrothiazolo-[3,2-a]pyridinium ring structure (II) had been synthesized by condensation of 1,2-dibromoethane with pyrid-2-thione.[3]

It was found that pyrid-2-thiones could be prepared from 3-hydroxy-2-bromo- or iodo-pyridines. The latter were obtained from 3-hydroxypyridines. Electrophilic substitution such as bromination of 3-hydroxypyridine (III) occurs in the 2-position [4] since strong electron donating groups such as the hydroxyl group will direct more strongly than the annular nitrogen. However, other isomeric brominated products are also formed as seen by chromatography. With the *p*-position to the hydroxyl group occupied as in 3-hydroxy-6-methyl-pyridine (IV) the substitution is more specific. Thus bromine in pyridine furnished the 2-bromo derivative (VI) in 70—80 % yield. The structure was

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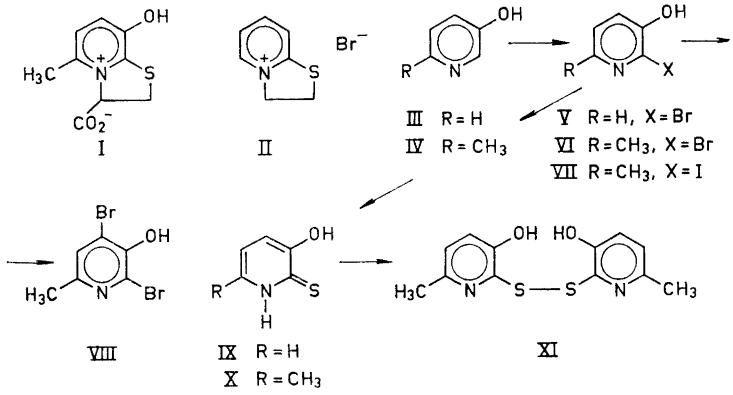
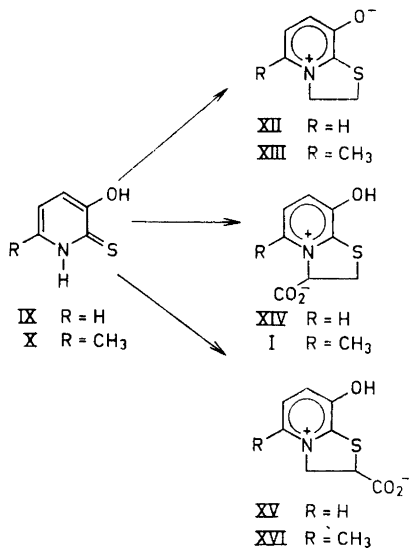


confirmed by the NMR spectrum in TFA; two adjacent aromatic protons resonating in an AB system at 1.93 and 2.35 τ (J=8.5 cps) with the loss of the lower field quartet at 1.87 τ due to the 2-proton in the starting material (IV). The use of two equivalents of bromine in pyridine led to a dibromo derivative as shown by an one aromatic proton singlet in the NMR spectrum at 2.04 τ. The second bromine atom must have entered the 4-position (VIII) due to the directive power of the hydroxyl group in 3-position. Further evidence for the correctness of structure VIII comes from the findings that nucleophilic substitution with potassium hydrogen sulphide could not easily be made to occur selectively in the 2-position. Therefore the second bromine is also activated towards nucleophilic substitution by the annular nitrogen and must be in the 4-position. The iodo analogue (VII) was obtained by treatment of IV with aqueous sodium iododichloride.

Heating the bromo- or iodo-pyridinols (V—VII) with excess potassium hydrogen sulphide in propylene glycol or DMF furnished the corresponding pyrid-2-thiones. The introduction of sulphur was also effected by the use of the sodium salt of thioacetic acid or by heating with thiourea. The vigorous reaction conditions required to make thiourea react with the bromo compound (VI) were such that the intermediate isothiuronium salt decomposed directly to the thione (X). Reactions on a small scale are best carried out in a nitrogen atmosphere to prevent oxidation to the corresponding disulphides. The disulphide (XI) was preparatively available by oxidation of the thiolactam (X) with bromine in ethyl acetate. The disulphide on the other hand, could be reduced back to the thiolactam by treatment with sodium borohydride in alcohol.

The thiolactam (X) was condensed with 1,2-dibromoethane in methanolic sodium methoxide and the 5-methyldihydrothiazolo[3,2-a]pyridinium-8-oxide (XIII) thus obtained was found to be identical with the decarboxylated material isolated from bovine liver hydrolysates.[1] The cyclisation could also be effected by heating in an inert solvent such as benzene without the addition of a base. The desmethyl analogue (XII) was obtained in the same way.

The sulphur atom is the strongest nucleophilic part of the thiolactam. The cyclisation must therefore be initiated by sulphur replacement of a



bromine atom followed by nucleophilic attack from the annular nitrogen onto the carbon carrying the other bromine atom. No *O*-alkylation has been observed. Although the cyclisation must proceed in two stages, we have not been able to isolate the intermediate (XVIII) since the conditions used to effect *S*-alkylation are such that the reaction will at once proceed further. However, the use of a potential leaving group on the β-carbon of the C_2-unit such as a hydroxyl group (XX) and conversion of this to the mesyl derivative (XXI) by treatment with mesyl chloride in cold pyridine has enabled us to isolate a such reactive intermediate. The mesyl derivative obtained was slightly contaminated with the cyclised product (XIII). On paper chromatography in an aqueous ammonia system the mesyl derivative was completely cyclised but no ring closure occurred in an acetic acid system. Preparatively, therefore, the cyclisation was carried out in cold aqueous ammonia or by heating in pyridine.

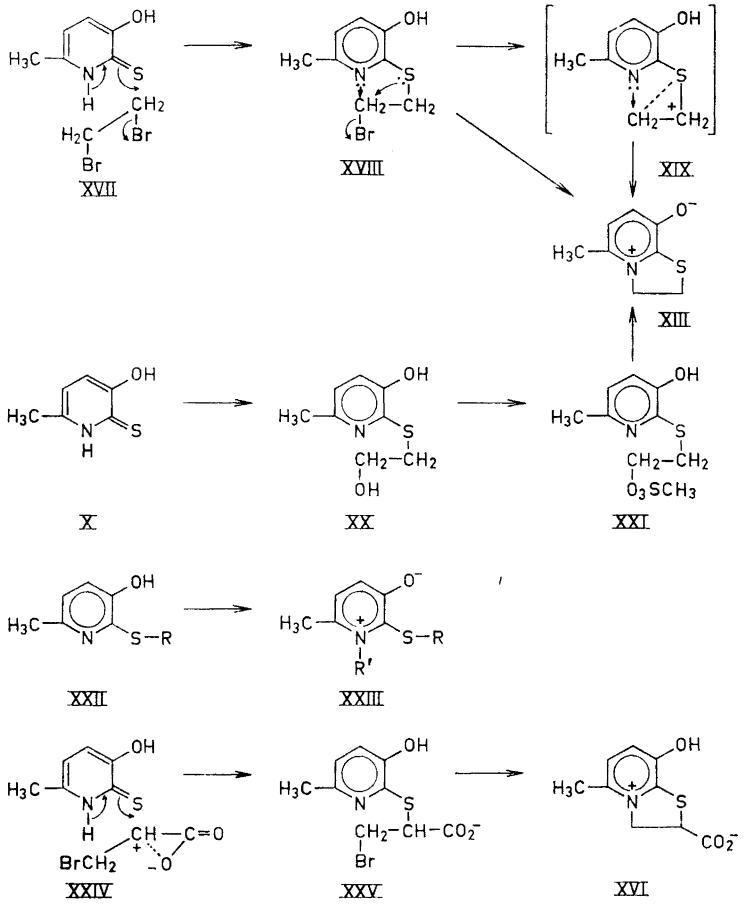
The hydroxyethioether (XX) was synthesized by alkylation of the thiolactam (X) as its anion in aqueous solution with ethylene bromohydrin. Alternatively the alkylation could be done by heating the thiolactam with the alkylating reagent in an inert solvent such as toluene in the absence of a base.

N-Quaternisation of 2-alkylthioethers (XXII) with alkyl halides requires vigorous conditions [5] although the electron donating properties of the thioether sulphur should favour *N*-quaternisation. The reason for this failure must be sought in the size of the thioether group which hinders the approach of the alkyl halide to the annular nitrogen.

The ease of cyclisation could be explained by sulphur neighbouring-group participation which becomes more important than the steric hindrance.

However, in this case the nucleophilic annular nitrogen would have to approach the electrophilic carbon from the same side as the participating sulphur (XIX). A more likely explanation therefore would be that the electrophilic carbon in the primary condensation product (XVIII) is correctly spaced for cyclisation to a favoured 5-membered ring.

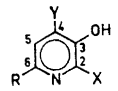
Unsymmetrically substituted 1,2-dibromoethane derivatives in the cyclisation reaction will give rise to two positional isomeric products in various proportions depending on the nature of the substituents and the experimental conditions used. Thus, treatment of the thiolactam (IX or X) with the methyl ester of 2,3-dibromopropionic acid in sodium alkoxide in the cold gave the 3-carbomethoxy derivatives. Acid hydrolysis furnished the desired



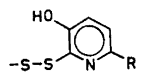
acids (I, XIV). The condensation product thus obtained from X was in all respects found to be the racemic equivalent of the levo rotatory product isolated from liver hydrolysates. The structure of the latter substance had

been postulated to be I from chemical degradation work.[1] The use of a weaker base such as potassium carbonate in alcohol or DMF lead to a mixture of the two possible isomers (I and XVI). In view of this the formation of the 3-isomer (I) could be due to a direct S_N2 displacement of the bromine atom on the β-carbon by the sulphur anion. However, since the 3-isomer was obtained almost exclusively in the experiment with the stronger base present a more likely explanation would be a beta elimination-addition process. On the other hand the sodium salt of 2,3-dibromopropionic acid in methanol furnished the 2-isomer (XVI) as expected for neighbouring-group activation by the carboxylate anion (XXIV). The same isomer was obtained by heating the thiolactam (X) with either 2,3-dibromopropionic acid or its methyl ester in an inert solvent such as benzene without added base. A similar carbonyl group participation mechanism could be in operation in these cases.

Table 1.

NMR spectra in TFA 

Substance	Substituents			Chemical shift in τ-values			Coupling constants in cps		
	R	X	Y	4	5	6	$J_{4,5}$	$J_{5,6}$	$J_{4,6}$
V	H	Br	H	1.83	2.12	1.70	8.5	5.5	2.0
VI	CH_3	Br	H	1.93	2.35	7.20	9.0	—	—
VII	CH_3	I	H	2.10	2.37	7.18	9.0	—	—
VIII	CH_3	Br	Br	—	2.04	7.22	—	—	—
IX	H	SH	H	2.07	2.40	1.90	8.5	6.0	1.5
X	CH_3	SH	H	2.22	2.65	7.30	8.5	—	—
XI	CH_3	[a]	H	1.90	2.33	7.15	9.0	—	—

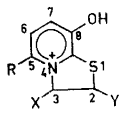
[a] 

The NMR data in TFA of the pyridines prepared are collected in Table 1. The expected upfield shift on substitution of halogen with sulphur is demonstrated. Perhaps unexpected is the marked downfield shift of the protons in the disulphide (XI) as compared to the thiolactam (X). In the desmethyl series (V, IX) the AB system of the methyl series has become an ABX system. The lowest chemical shift (1.70 τ) found in the spectrum of V must be assigned

to the proton attached to the carbon next to the annular nitrogen. This is coupled fairly strongly, $J=6$ cps, to its neighbouring AB proton ($J=8.5$ cps) resonating at 2.12 τ. Its coupling constant to the other AB proton at C-4 is about 2 cps. The chemical shifts for the protons in the thione (IX) are deduced in the same way.

Table 2.

NMR spectra in TFA



Substance	Substituents			Chemical shift in τ-values					Coupling constants in cps			
	R	X	Y	2	3	5	6	7	$J_{2,3}$	$J_{5,6}$	$J_{6,7}$	$J_{5,7}$
XII	H	H	H	6.08	4.73	1.78	2.47	2.25	8.0	6.0	8.5	<1
XIII	CH_3	H	H	6.12	4.90	7.25	2.70	2.30	8.0	—	8.5	—
XIV	H	CO_2H	H	[a]5.72	3.75	1.65	2.41	2.15	—	6.0	8.5	<1
I	CH_3	CO_2H	H	[a]5.73	3.67	7.22	2.58	2.15	—	—	8.5	—
XV	H	H	CO_2H	[b]4.13—5.07		1.70	2.37	2.15	—	6.0	8.5	1.0
XVI	CH_3	H	CO_2H	[b]4.24—4.89		7.17	2.60	2.17	—	—	8.5	—

[a] The center of a multiplet due to the 2 C_2-protons. [b] Methylene and methine protons fall in the same region.

Table 2 shows the NMR data of the quaternary derivatives in TFA. Again the chemical shifts of the aromatic protons can be deduced from the coupling constants in the desmethyl series, *e.g.* in XII. The protons in the aromatic ABX system resonate at 1.78, 2.25, and 2.47 τ. The low field proton is coupled to the proton at the highest field with a coupling constant of about 6, while the other coupling constant is less than one. Therefore, as in the case of the simple pyridines above, the H_7-proton has the lower chemical shift of the AB-protons. The methyl group (XIII) pushes the chemical shift of the H_6-proton about 0.15 τ towards higher field as compared to the desmethyl series. The H_7-protons are not affected. The methine-methylene protons in the dihydrothiazolo ring resonate in a collapsed ABX system. In the 3-carboxy series the methine proton next to the quaternary nitrogen atom is found as a quartet at 3.7 τ and the methylene protons next to the sulphur as a triplet centered at 5.7 τ. The methylene protons adjacent to the quaternary nitrogen are found at a lower field than when adjacent to the sulphur. In the 2-carboxy series the chemical shifts for the methylene and the methine proton therefore

become nearly the same, the 3 protons appearing in an unresolved multiplet at 4.2—5.0 τ.

The pyridinium derivatives are very strongly blue fluorescent in UV light. The members of the 5-methyl series (XIII, I, XVI) have the longwave absorption band in 0.1 N NaOH aq. at 360 mμ. In 0.1 N HCl aq. this band is found at 340 mμ, which corresponds to the expected phenolate shift caused by the 8-hydroxy group. The corresponding long-wave absorption bands in the desmethyl series (XII, XIV, XV) are found at 350 and 330 mμ, respectively.

The 3-carboxy derivatives in the solid state show strong carboxylate absorption at 1640 cm^{-1}. In the 2-carboxy series the absorption is at 1620 cm^{-1} which agrees better with the usual range quoted for such absorption. The rather high value for the 3-carboxy derivative is caused by the quaternary nitrogen and corresponds to what is found for amino acids present as zwitterions.

EXPERIMENTAL

Paper chromatography and TLC on silica gel in BuOH:EtOH:NH_3:H_2O (4:1:2:1) and BuOH:HOAc:H_2O (100:22:50) were used in this work. The UV data were recorded on a Beckmann DB—G UV spectrometer. The extinction coefficients are quoted as log ε. The NMR spectra were recorded on a Varian A-60 spectrometer and the IR spectra on a Perkin-Elmer 237 IR spectrophotometer.

2-Bromo-3-hydroxy-6-methylpyridine (VI). 2-Methylpyridine-5-sulphonic acid was prepared by sulphonation of α-picoline by the method of McElvain and Goose.[6] 5-Hydroxy-2-methylpyridine was then prepared by fusion of 2-methylpyridine-5-sulphonic acid with potassium hydroxide by the method of Marion and Cockburn.[7]

3-Hydroxy-6-methylpyridine (4.4 g, 0.04 mole) was dissolved in dry pyridine (80 ml) and a solution of bromine (7.05 g, 0.44 mole) in dry pyridine (40 ml) added dropwise over 10 min to the stirred solution at room temperature. The reaction mixture was stirred for an hour at room temperature, evaporated at reduced pressure, the residue triturated with water (30 ml), the white-grey solid filtered off, washed with water and dried; yield 5.5 g (74 %); white crystals from ethanol, m.p. 187—189°. (Found: C 38.44; H 3.10; Br 42.58; N 7.47. Calc. for C_6H_6BrNO: C 38.42; H 3.22; Br 42.50; N 7.45).

IR (KBr). Strongly H-bonded OH group in the region 2600—3000 cm^{-1} characterised by a number of peaks.

λ_{max} 0.1 N NaOH: 310 (3.77), 245 mμ (4.05); 0.1 N HCl: 300 (3.97), 235 mμ (3.78).

2-Bromo-3-hydroxypyridine (V). This compound was prepared from 3-hydroxypyridine.[4] λ_{max} 0.1 N NaOH: 305 (3.84), 240 mμ (3.94); 0.1 N HCl: 295 (3.81), 225 mμ (inversion).

2,4-Dibromo-3-hydroxy-6-methylpyridine (VIII). Bromine (35.4 g, 0.22 mole) in pyridine (200 ml) was added dropwise over 30 min to a stirred solution of 3-hydroxy-6-methylpyridine (11.0 g, 0.1 mole) in pyridine (300 ml) at room temperature. The temperature in the reaction mixture rose to 40° during the bromine addition. After standing overnight at room temperature the precipitated pyridine hydrobromide was removed by filtration, the filtrate concentrated to about 150 ml, more pyridine hydrobromide removed, the filtrate evaporated to dryness, the reddish brown residue dissolved in ethanol (50 ml) and conc. HCl (20 ml) added. On standing in the cold the dibromo derivative slowly crystallized out. Some co-precipitated pyridine salt was removed by trituration with water. There remained 21.5 g (80 %) of the desired substance, m.p. 102—106°. This material could be further purified by sublimation or recrystallization from ligroin. The analytical sample melted at 107—109°. (Found: C 27.00; H 1.86; N 5.31; Br 60.34. Calc. for $C_6H_5Br_2NO$: C 27.00; H 1.90; N 5.25; Br 59.87). λ_{max} 0.1 N NaOH: 315 (3.86) and 240 mμ (3.80); 0.1 N HCl: 290 mμ (3.78).

3-Hydroxy-2-iodo-6-methylpyridine (VII). Aqueous 3.5 N sodium iododichloride (15.7 ml) was added dropwise to pyridine (50 ml) with stirring at room temperature. The resultant suspension was added portionwise over 30 min to a stirred solution of 3-hydroxy-

6-methylpyridine (5.5 g, 0.05 mole) dissolved in pyridine (150 ml). After standing at room temperature overnight aqueous 2 % sodium pyrosulphite solution was added dropwise at room temperature until the solution was decolorised. The solution was then evaporated to dryness at reduced pressure, the residue suspended in water (30 ml) and hydrochloric acid (10 ml) added. The white crystalline solid formed (6.2 g 53 %), m.p. 173°, had m.p. 174° (subl.) after recrystallization from ethanol. (Found: C 30.39; H 2.42; N 6.23. Calc. for C_6H_6INO: C 30.66; H 2.57; N 5.96).

IR(KBr). Strong H-bonded OH group in the 2600—3000 cm^{-1} region. λ_{max} 0.1 N NaOH: 320 (3.87), 245 mμ (4.01); HCl: 315 (4.10), 225 mμ (inversion).

3-Hydroxy-6-methylpyrid-2-thione (X). a) Potassium hydrogen sulphide (14.4 g, 0.2 mole) was added to dry propylene glycol (250 ml) and the stirred mixture heated to boiling when the solid dissolved. The temperature was lowered to 160° and 2-bromo-3-hydroxy-6-methylpyridine (12.0 g, 0.064 mole) added in small portions over 15 min. The greenish solution was stirred under reflux for 20 h and the glycol removed at reduced pressure at 100°. The residue was dissolved in water (150 ml), treated with charcoal, and the solution slowly acidified with 50 % acetic acid (30 ml). After standing in the cold overnight the yellow solid (7.3 g, 83 %), m.p. 170—175°, was collected and recrystallized from ethanol, m.p. 174—177°. (Found: C 51.07; H 5.00; N 9.93; S 22.73. Calc. for C_6H_6OS: C 51.09; H 5.14; N 9.91; S 22.61).

λ_{max} 0.1 N NaOH: 370 (3.82), 265 (3.71), 240 mμ (3.76); 0.1 N HCl: 355 (410), 260 mμ (3.78).

b) 2-Bromo-3-hydroxy-6-methylpyridine (3.0 g, 0.016 mole) and the sodium salt of thioacetic acid (4.5 g, 0.045 mole) in anhydrous DMF (35 ml) were heated at 130° overnight, allowed to cool, the insoluble material removed by filtration and the filtrate concentrated to a small volume and poured into water. The desired thione was slowly precipitated as yellow needles; 0.5 g (22 %), m.p. 172—176°. One recrystallization from water gave m.p. 175—177°.

c) From thiourea: 2-Bromo-3-hydroxy-6-methylpyridine (1.9 g, 0.01 mole) and thiorea (1.5 g, 0.02 mole) were heated together in propylene glycol (75 ml) at 160° for 24 h. The solution was then evaporated at reduced pressure, the residue dissolved by heating in ethanol (5 ml) and the hot solution treated with a little charcoal. Yellowish solid was precipitated on cooling (0.53 g, 37 %), m.p. 167—180°. After recrystallization from ethanol the yellow needles melted at 167—175°. The physical data for this product were the same as for the above prepared thione. Therefore the isothiuronium salt originally formed must have been decomposed to the desired thione under the vigorous experimental conditions employed.

3-Hydroxypyrid-2-thione (IX). Potassium hydrogen sulphide (18 g, 0.25 mole) was dissolved in propylene glycol (250 ml) by heating to 160°. The temperature of the green solution was lowered to 90° and 2-bromo-3-hydroxypyridine (17.4 g, 0.10 mole) added in small portions over 15 min. The mixture was stirred at 120° for 24 h, evaporated to dryness at reduced pressure, the residue dissolved in water (40 ml), and the solution acidified with 50% acetic acid (pH ~5) when the yellow solid (10.2 g, 80%) was precipitated. Recrystallization from ethanol gave m.p. 144—145°. (Found: C 47.32; H 3.86; N 11.14; S 25.01. Calc. for C_5H_5NOS: C 47.22; H 3.96; N 11.02; S 25.22).

λ_{max} 0.1 N NaOH: 355 (3.91), 2.65 mμ (3.80); 0.1 N NCl: 350 (4.02), 260 mμ (3.77).

3,3'-Dihydroxy-6,6'-dimethyl-2,2'-dipyridyldisulphide (XI). Bromine (32 g, 0.2 mole) dissolved in ethyl acetate (460 ml) was added dropwise (90 min) to a stirred solution of 3-hydroxy-6-methylpyrid-2-thione (28.2 g, 0.2 mole) in ethyl acetate (900 ml) in the cold. The solid formed (49.3 g) melted at 235—237° after recrystallization from ethanol. The free base was obtained by dissolving the hydrobromide (39 g) in water (800 ml) and neutralizing with sodium bicarbonate; white solid (14.3 g, 58 %), m.p. 207°. An analytical sample recrystallized from ethanol had m.p. 209—210°. (Found: C 51.37; H 4.57; N 9.97; S 21.97. Calc. for $C_{12}H_{12}N_2O_2S_2$: C 51.40; H 4.31; N 9.98; S 22.28). Dissolved in isopropanol the disulphide was readily cleaved by sodium borohydride as shown by chromatography.

λ_{max} 0.1 N NaOH: 335 (3.96), 240 mμ (4.12); 0.1 N HCl: 345 (3.95), 310 mμ (3.94).

5-Methyldihydrothiazolo [3,2-a]pyridinium-8-oxide (XIII). a) 3-Hydroxy-6-methylpyrid-2-thione (1.42 g, 0.01 mole) was added to absolute ethanol and a 2.7 M methanolic sodium methoxide solution (8.15 ml, 0.022 mole) added. To this solution at room temperature was added dropwise 1.2-dibromoethane (2.07 g, 0.011 mole). The solution

was stirred at room temperature for 20 h. In another experiment the alkylation was run at 60° and was then complete after 6 h.

After evaporation of the solvent the residue was extracted with boiling isopropanol (2 × 30 ml) and the extracts again evaporated to dryness. The residue was redissolved in boiling ethanol, a little undissolved material filtered off, and the filtrate concentrated to the point of crystallization. After standing in the cold the white crystalline material (0.91 g), m.p. 202—205°, was collected. Further concentration of the filtrate furnished another crop (0.82 g), (total yield hydrated material = 90 %). Further recrystallization from methanolic or ethanolic acetone gave m.p. 220° (decomp.): (Found: C 57.73; H 5.37; N 8.42; S 19.07. Calc. for C_8H_9NOS: C 57.46; H 5.43; N 8.37; S 19.17).

λ_{max} 0.1 N NaOH: 360 (4.02), 245 mμ (3.89); 0.1 N HCl: 340 (3.98), 240 mμ (3.70).

b) 3-Hydroxy-6-methylpyrid-2-thione (3.0 g, 0.021 mole) was dissolved in boiling benzene (50 ml) and 1,2-dibromoethane (4.8 g, 0.026 mole) added. The resulting solution was refluxed overnight, allowed to cool and the white crystalline material filtered off (4.0 g, 51 %) and recrystallized from methanol. This substance started to sublime at 290° and decomposed above 300°. The zwitterion was obtained by addition of one equivalent of an inorganic base to a concentrated solution of the hydrobromide. Alternatively an aqueous solution of the hydrobromide was passed through a DEAE-Sephadex column in the free amine form and the combined aqueous eluates concentrated to a small volume until the zwitterion started crystallizing out.

c) 2-β-Hydroxyethio-3-hydroxy-6-methylpyridine (1.86 g, 0.01 mole) was dissolved in pyridine (25 ml) and a solution of mesylchloride (1.25 g, 0.011 mole) added very slowly to the cold, stirred solution. A white crystalline solid was slowly precipitated. The reaction mixture was stirred in the cold for 1 hour and the solid mesyl derivative filtered off; yield 2.2 g (83.0 %), m.p. 108—114°. On paper chromatography in the ammonia system the product had the same R_F value as the title compound and otherwise behaved as such. Cyclisation therefore had occurred during chromatography. This was not true in the acetic acid system.

1.0 g of the product was therefore dissolved in methanol (10 ml) and aqueous ammonia (1 ml) added. Chromatography showed that the cyclisation was complete after standing in the cold for 30 min. The solution was evaporated, the residue dissolved in water (3 ml) and acetone (2 ml) added. White needles of the title compound were slowly precipitated, m.p. 220—225°.

The cyclisation was also carried out by heating the mesyl derivative as first formed in pyridine at 80° for 2 h.

2-β-Hydroxyethio-3-hydroxy-6-methylpyridine (XX). a) Ethylene bromohydrin (56.3 g, 0.45 mole) was added dropwise over 20 min at 40° to a vigorously stirred solution of 3-hydroxy-6-methylpyrid-2-thione (42.3 g, 0.3 mole) in N NaOH aq. (450 ml, 0.45 mole). The solution was kept at 40° for another 2 h, then allowed to stand at room temperature overnight. A little semisolid precipitate formed was removed by filtration and the pH of the filtrate adjusted to 7.6. The yellowish oil formed was extracted into ether, the extracts washed, dried and evaporated leaving the desired substance (44.8 g, 81 %), m.p. 92—100°. Recrystallization from water gave white crystals, m.p. 102—104°. (Found: C 51.87; H 5.99; N 7.56; S 17.31. Calc. for $C_8H_{11}NO_2S$: C 51.52; H 6.07; N 5.77; S 17.18). In a similar experiment with ethylene chlorohydrin the yield was 94 %.

b) A solution of ethylene chlorohydrin (2.35 g, 0.03 mole) and 3-hydroxy-6-methylpyrid-2-thione (2.82 g, 0.02 mole) in dry toluene (50 ml) was refluxed for 48 h. The solid precipitate formed was filtered form the hot solution; yield 1.9 g (43 %), m.p. 122—134°. The hydrochloride thus obtained could be recrystallized from water or ethanol, m.p. 135—141°.

The free base was obtained by neutralization of an aqueous solution with sodium hydroxide.

λ_{max} 0.1 N NaOH: 325 (3.88), 245 mμ (3.75); 0.1 N NCl: 330 (3.94), 240 mμ (3.53).

Dihydrothiazolo[3,2-a]pyridinium-8-oxide (XII). To 3-hydroxypyrid-2-thione (20 g, 0.16 mole) in methanol (150 ml) was added a solution of sodium methoxide (13.0 g, 0.24 mole). To the resultant solution was added dropwise with stirring at 0° 1,2-dibromoethane (31.0 g, 0.16 mole). The reaction mixture was then stirred for 2 days at room temperature, evaporated, the residue extracted with boiling isopropanol (3 × 300 ml), the insoluble salt discarded, the isopropanol evaporated, the residue dissolved in water (400 ml), the pH adjusted to 7 and the solution extracted with ether (3 × 150 ml) to

remove unreacted thiolactam. Slow concentration of the solution (pH 4 with HCl) precipitated the hydrochloride (22.0 g, 62 %). This material (15.0 g), being contaminated with a little unreacted thione, was dissolved in water (200 ml), the pH adjusted to 7.4 and the solution extracted with ethyl acetate (3 × 75 ml), the solution treated with a little charcoal, the pH adjusted to about 3 and the solution concentrated to about 50 ml. The white hydrochloride (12 g) crystallized out. The free base was obtained by dissolving the hydrochloride in water, passing this solution through a DEAE-Sephadex column in the free amine form, and concentrating the combined aqueous eluates until the zwitterion started crystallizing out. The white solid could be recrystallized from isopropanol, m.p. 150—158°. (Found: C 54.73; H 4.82; N 9.05. Calc. for C_7H_7NOS: C 54.91; H 4.61; N 8.15).

λ_{max} 0.1 N NaOH: 350 (3.98), 245 mμ (3.84); 0.1 N HCl: 330 (391), 230 mμ (3.68).

8-Hydroxy-5-methyldihydrothiazolo[3,2-a]pyridinium-3-carboxylate (I). 2.7 M Methanolic sodium methoxide (8.2 ml, 0.022 mole) was added to 3-hydroxy-6-methylpyrid-2-thione (1.4 g, 0.01 mole) in dry methanol (50 ml). The yellow solution was warmed to 40° and methyl 2,3-dibromopropionate (2.86 g, 0.012 mole) added dropwise with stirring over 15 min. After the addition was completed the reaction mixture was stirred for another 2 h at 40° and then evaporated to dryness at reduced pressure. The residual material was extracted with methylene chloride (30 ml), the insoluble material removed by filtration and the filtrate evaporated. A solution of the residue in acetone (5 ml) slowly deposited the ester (1.30 g, 58 %), m.p. 140—145°.

The methyl ester was hydrolysed to the acid in 2 N HCl aq. by heating at 90° for 2 h. The solution was then concentrated to a small volume and the pH adjusted to 3.5—4 with alkali. On standing in the cold the zwitterion was slowly precipitated, m.p. 160—162°. The melting point remained constant on recrystallization from water. (Found: S 15.23. Calc. for $C_9H_9NO_3S$: S 15.18). This material had the same blue fluorescence as the substance isolated from liver. It had the same R_F values in chromatography. The same UV spectrum as given below. The same NMR spectrum. The IR spectrum was characterised by carboxylate absorption at 1640 cm^{-1}.

λ_{max} 0.1 N NaOH: 360 (4.05), 245 mμ (4.01); 0.1 N HCl: 340 (4.01), 240 mμ (3.85).

8-Hydroxydihydrothiazolo[3,2-a]pyridinium-3-carboxylate (XIV). Prepared as above from 3-hydroxypyrid-2-thione, m.p. 180° (decomp.). (Found: C 48.90; H 3.85; N 6.95. Calc. for $C_8H_7NO_3S$: C 48.72; H 3.58; N 7.10).

IR (KBr). Broad carboxylate absorption at 1640 cm^{-1}.

λ_{max} 0.1 N NaOH: 350 (4.01), 245 mμ (3.96); 0.1 N HCl: 330 (3.96), 230 mμ (3.87).

8-Hydroxy-5-methyldihydrothiazolo[3,2-a]pyridinium-2-carboxylate (XVI). a) 3-Hydroxy-6-methylpyrid-2-thione (5.0 g, 0.036 mole) was dissolved in benzene (180 ml) and about 30 ml of the solution distilled off to remove any water present. Methyl 2,3-dibromopropionate (15 g, 0.061 mole) dissolved in benzene (20 ml) was added dropwise over 1 h to the refluxing solution. After heating under reflux for 60 h the solid precipitate (8.13) was collected by filtration. This material was dissolved in water (200 ml), some insoluble material removed by filtration and 2 N NaOH aq. (20 ml, 0.04 mole) added dropwise over 1 h at 60°. After stirring at 60° overnight the solution had pH 6. The cold solution was extracted with ether to remove any unchanged thiol. The solution was then concentrated to the point of crystallization and left in the cold. The yield was 3.0 g of a white crystalline solid. Further concentration of the filtrate furnished another 0.26 g of the same material, *i.e.* total yield 3.3 g (54 %). Recrystallization from acetic acid gave unsharp m.p. 270° (decomp.). (Found: C 47.57; H 4.75; N 6.28. Calc. for $C_9H_9NO_3S—H_2O$: C 47.15; H 4.84; N 6.11).

IR (KBr). Broad band at 1620 cm^{-1} due to carboxylate group.

λ_{max} 0.1 N NaOH: 360 (4.02), 245 mμ (3.99); 0.1 N HCl: 340 (4.00), 240 mμ (3.83).

b) When 2,3-dibromopropionic acid was used instead of the ester the same product was obtained.

c) 3-Hydroxy-6-methylpyrid-2-thione (1.4 g, 0.01 mole) was dissolved in methanolic (10 ml) sodium methoxide (from 0.46 g of Na, 0.02 M) and 2,3-dibromopropionic acid (2.3 g, 0.01 M) added. The solution was left at room temperature for 2 days and then placed at 0° when the title compound crystallized out. Recrystallization from water gave m.p. about 263° (decomp.).

8-Hydroxydihydrothiazolo[3,2-a]pyridinium-2-carboxylate (XV). To a solution of 3-hydroxypyrid-2-thione (12.7 g, 0.1 mole) in benzene (200 ml) and methanol (100 ml) was added methyl 2,3-dibromopropionate (25.0 g, 0.1 mole). The solution was refluxed

for 2 days, evaporated to dryness and the residue dissolved in water (300 ml) by addition of dilute NaOH to pH 11. After standing overnight the pH was brought to 7, unreacted solid thione filtered off, the filtrate extracted with ethyl acetate (3 × 100 ml), the aqueous phase decolorised with charcoal and evaporated to dryness. The residue was extracted with hot methanol, the methanol extracts evaporated and the residue dissolved in boiling water (175 ml). A white crystalline material (5 g, 33 %) was precipitated on cooling, m.p. 250° (decomp.). (Found: C 47.98; H 3.92; N 7.43; S 15.86. Calc. for $C_8H_7NO_3S \cdot \frac{1}{2}H_2O$: C 48.35; H 4.56; N 7.05; S 16.14).

λ_{max} 0.1 N NaOH: 350 mμ (4.00), 245 mμ (3.79); 0.1 N HCl: 330 mμ (3.96), 230 mμ (3.76).

IR (KBr). Broad and strong bond at 1610 cm^{-1} due to carboxylate group.

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