MIMOSINE, MIMOSINAMINE AND 3,4-DIHYDROXYPYRIDINE

Noel K.Hart, Albert Hofmann, John A.Lamberton* and Colin M.Richards
Division of Applied Organic Chemistry, C.S.I.R.O., P.O. Box 4331,
Melbourne, Victoria, Australia. 3001.

A new extraction method has made mimosine easily available. (±)-Alanine formed in the acid hydrolysis of mimosine appears to be a secondary product derived from pyruvic acid by a transamination reaction. Alkylation and acylation reactions of 3,4-dihydroxypyridine are described and mimosinamine has been prepared in low yield.

Recent interest in mimosine (I) as a chemical de-fleecing agent[1] led us to seek a better method for the isolation of mimosine from seeds of Leucaena leucocephala. Although the mimosine content is high the original method[2] of heating seed meal with water gives a thick viscous mass from which mimosine is isolated only with difficulty, and the dialysis method[3] which gives pure mimosine in high yield is impracticable on a large scale. The following simple large scale method has now been devised. Seed meal (20 kg) was allowed to imbibe water (40 l) overnight. Then methanol

(160 l) containing ammonia (0.880; 5 l) was added and the suspension stirred (2 hr). Evaporation of the methanolic extract gave almost pure crystalline mimosine, and after a single repetition of the extraction with more methanol (160 l) and ammonia (0.880; 5 l) a good recovery of mimosine (~560 g) was obtained. It is important to avoid heating the extract; enough methanol must be added to suppress the solubility of troublesome mucilaginous material, while ammonia is needed to dissolve mimosine which has low solubility in water and methanol.

In dilute HCl (0.1 N) at reflux temperature mimosine undergoes hydrolysis to give 3,4-dihydroxypyridine (II) and pyruvic acid.[4] Recovery of 3,4-dihydroxypyridine is only in low yield (~40%), and pyruvic acid, isolated by continuous distillation from the reaction flask and conversion into its 2,4-dinitrophenylhydrazone, is in corresponding low yield. Although the remaining mixture of products contains no serine, it does contain alanine (13% yield). It seems mechanistically improbable that alanine should be formed directly from mimosine, and it is more likely to come from a transamination through the intermediate (III) derived from pyruvic acid. In accordance with the transamination route to alanine, it has been shown that alanine produced by hydrolysis of mimosine in the absence of 2,4-dinitrophenylhydrazine is racemic. This route has been confirmed by adding 2,4-dinitrophenylhydrazine to the reaction mixture so that pyruvic acid is trapped as the insoluble 2,4-dinitrophenylhydrazone, and the formation of further products is prevented.

Typically mimosine (19.8 g) and 2,4-dinitrophenylhydrazine (29.7 g) were heated at reflux temperature in HCl (0.2 N, 1200 ml). After 24 hr crude insoluble pyruvic acid 2,4-dinitrophenylhydrazone was filtered off, purified by extraction into $NaHCO_3$ (5%, 200 ml), and after removal of excess 2,4-dinitrophenylhydrazine by extraction ($CHCl_3$), precipitated by addition of conc. HCl (yield, 25.7 g, 96%). The original solution of reaction products was also washed repeatedly ($CHCl_3$) to remove unreacted 2,4-dinitrophenylhydrazine, then evaporated to dryness, taken up in water (60 ml), and applied to Zeocarb 225 (H^+, 250 ml). After elution with distilled water (2.2 l), 3,4-dihydroxypyridine was eluted with aqueous NH_3 (2M, 2 l), and pure crystalline 3,4-dihyroxypyridine (10.85 g, 98%) was obtained on evaporation. No alanine was detectable under these conditions.

Because of the accessibility of mimosine and 3,4-dihydroxypyridine by these methods it is of interest to explore the possible preparation of mimosinamine (IV) from 3,4-dihydroxypyridine, as an alternative to the synthetic route from kojic acid through 3-benzyloxy-4-pyrone.[5] Reaction of 3,4-dihydroxypyridine with benzyl chloride (1 equiv) and NaOEt (2 equiv) in refluxing ethanol afforded N-benzyl-3,4-dihydroxypyridine (V) in 38% yield, m.p. 219-220.5° from ethanol, Fe^{+++} positive, p.m.r. ($CDCl_3$-CD_3OD) δ 5.18 (s, 2H, $-CH_2N$); δ 6.5 (d, 1H, $J_{5,6}$ 6 Hz, H5); δ 7.35 (m, 5H, phenyl); δ 7.55 (d, 1H, $J_{2,6}$ 2Hz, H2); δ 7.65 (dd, 1H, H6); m/e (e.i.) 201 (M^+, 68%), 91 (100). Found: C, 71.6; H, 5.5; N, 6.7. $C_{12}H_{11}NO_2$ requires C, 71.6; H, 5.5; N, 7.0%. Similarly from benzyl bromide (4 equiv), NaOEt (2 equiv) and 3,4-dihydroxypyridine (1 equiv) in ethanol or DMF, the N,O-dibenzyl derivative (VI) was isolated by chromatography

on neutral alumina in 55% and 41% yield respectively (m.p. 155-156° from acetone; p.m.r. ($CDCl_3$) δ 4.82 (s, 2H, O-CH_2); δ 5.15 (s, 2H, N-CH_2); δ 6.42 (d, 1H, $J_{5,6}$ 7 Hz, H5); δ 7.0 - 7.6 (m, 12H); m/e (e.i.) 291 (M^+, 46%), 200 (17), 185 (38), 91 (100). Found: C, 78.2; H, 5.8; N, 4.8%. $C_{19}H_{17}NO_2$ requires C, 78.4; H, 5.8; N, 4.8%. Hydrogenation of the dibenzyl compound (VI) over 5% Pd/C in methanol afforded the N-benzyl compound (V) in 62% yield. Reaction of a solution of Na (1.84 g) in EtOH (100 ml) with a suspension of 3,4-dihydroxypyridine (4.5 g) and 1-bromo-5-phthalimidopentane (6.6 g) in DMF (150 ml) for 24 hr at room temperature gave 1-(3-hydroxy-4-oxopyridin-1-yl)-5-phthalimidopentane (VII), 640 mg, m.p. 189-191° from ethanol, p.m.r. ($CDCl_3$) δ 1.2 - 2.0 (m, 6H), δ 3.80 (t, 4H, 2 x CH_2-N); δ 5.70 (s, 1H, OH); δ 6.4 (d, 1H, $J_{2,3}$ 7 Hz, H2), δ 7.25 (m, 2H, H4, H6); δ 7.80 (m, 4H); m/e (e.i.) 326 (M^+, 92%), 160 (100). Found: C, 66.2; H, 5.5; N, 8.2%. $C_{18}H_{18}N_2O_4$ requires C, 66.2; H, 5.5; N, 8.6%. Under similar conditions 4-pyridone afforded 1-phthalimido-5-(4-oxopyridin-1-yl)pentane (VIII) in 42% yield, m.p. 116-117° from ethanol-diethyl ether, M^+ 310.

1-(3-Hydroxy-4-oxopyridin-1-yl)-2-phthalimidoethane (IX) has been made by several methods, all of which give a low yield. Reaction of the sodium salt of 3,4-dihydroxypyridine with 1-bromo-2-phthalimidoethane in DMF, in DMF/ethanol (1:1), or in ethanol at various temperatures between 20° and reflux temperature, afforded (IX) in 2.8, 4.5, and 3.3% yields respectively. The N,O-dialkylated pyridone (X) was isolated in 2.9% yield from the DMF reaction, m.p. 206-207.5° from ethanol, m/e (e.i.) 457 (M^+, 2%). Found: C, 65.3; H, 4.3; N, 9.2%. $C_{25}H_{19}N_3O_6$ requires C, 65.6; H, 4.2; N, 9.2%. Other methods, K_2CO_3 in acetone, KOH

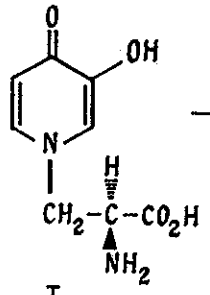
in toluene, and t-BuOK in acetone, all at reflux temperature, gave yields of 0.4, 0.3, and 1.6% respectively. Crystallization from ethanol-toluene gave (IX) as colourless cystals, m.p. 291-296°, m/e (e.i.) 284 (M^+, 100%), 174 (58), 60 (45). Found: C, 63.4; H, 4.4: N, 10.0%. $C_{15}H_{12}N_2O_4$ requires C, 63.4; H, 4.2; N, 9.9%. Reaction of (IX) with hydrazine hydrate in ethanol at reflux temperature for 25 minutes followed by hydrolysis of the intermediate product (6N HCl, 100°, 1 hr) gave mimosinamine in quantitative yield after isolation by ion-exchange chromatography.

Acylation of 3,4-dihydroxypyridine (II) has been studied initially as a means of purifying crude (II) isolated by hydrolysis of mimosine, although this method was not needed after development of the hydrolysis method given above. Acetylation of (II) with Ac_2O/pyridine, evaporation, and chromatography on neutral alumina gave a crystalline product m.p. 132-134°, shown to be the 1,3-diacetyl derivative (XI) by the p.m.r. spectrum ($CDCl_3$) which has two sharp 3H singlets in 1:1 ratio at δ 2.35 (OAc) and δ 2.65 (NAc), and signals at δ 6.45 (d, 1H, $J_{5,6}$ 7 Hz, H5), δ 8.10 (dd, 1H, $J_{2,6}$ 2 Hz, H6) and δ 8.20 (d, 1H, H2). If the product of acetylation is instead distilled under vacuum (120°, 0.08 mm) it is obtained as a clear viscous oil, and immediate examination by p.m.r. ($CDCl_3$) revealed NAc and OAc signals in 1:4 ratio indicating approximately 50% predominance of the O,O-diacetyl isomer (XII) over the N,O-diacetyl isomer (XI). The viscous liquid crystallized over a period of several hr at room temperature, and after 18 hr the p.m.r. spectrum indicated an equilibrium of O,O-diacetyl compound (XII) to N,O-diacetyl compound (XI) of 1:11. The equilibrium was displaced in favour of the O,O-diacetyl

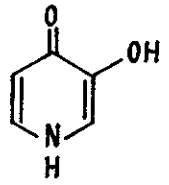
compound by holding the mixture above the melting point of the N,O-diacetyl compound. The rapid hydrolysis of N,O-acetyl compound (XI) in d_4-methanol was followed by p.m.r. spectroscopy. In $CDCl_3$-CD_3OD (1:1) $t_{\frac{1}{2}}$ was shown to be ≈10 min for the reaction N,O-diacetyl compound to 3-O-acetyl compound (NAc ——→ NH), while $t_{\frac{1}{2}}$ was ≈65 min for the reaction C3-OAc ——→ C3-OH. The ease of hydrolysis of the N-acetyl group was expected from earlier work on N-acetyl-4-pyridone,[6] but such ready loss of the 3-O-acetyl group was not expected. In a study of the acetylation of amines and phenols by reaction with 2-acetoxypyridine and 3-acetoxypyridine it was found that 3-acetoxypyridine is much less reactive.[7]

3-Benzoyloxy-4-pyridone (XIII) is considerably more stable in methanol than the 3-O-acetyl compound. After 1.5 hr in $CDCl_3$-CD_3OD, hydrolysis of (XIII) to 3,4-dihydroxypyridine could not be detected by p.m.r.

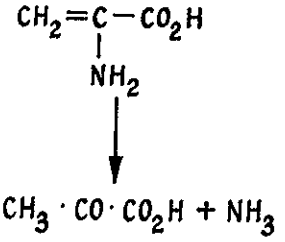
3-Benzoyloxy-4-pyridone was prepared from 3,4-dihydroxypyridine by reaction with benzoyl chloride in pyridine. Crystallization of the product from ethanol gave (XIII) as pale yellow crystals m.p. 185.5-188°, m/e (e.i.) 215 (M^+, 5%), 105 (100%); ν max. (KBr) 1655, 1755 cm^{-1}. Found: C, 66.7; H, 4.5; N, 6.6%. $C_{12}H_9NO_3$ requires C, 67.0; H, 4.2; N, 6.5%.

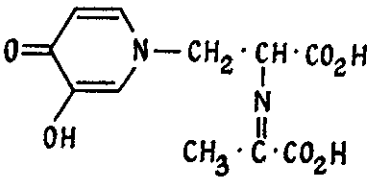


I

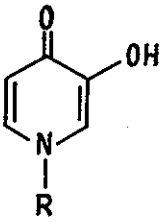


II



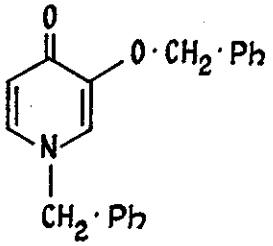


III

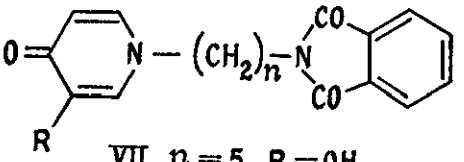


IV R = $CH_2 \cdot CH_2 \cdot NH_2$

V R = $CH_2 \cdot Ph$



VI

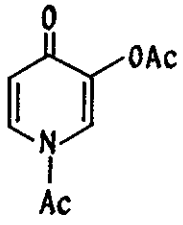


VII $n = 5$, R = OH

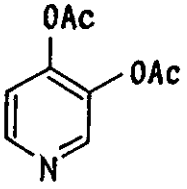
VIII $n = 5$, R = H

IX $n = 2$, R = OH

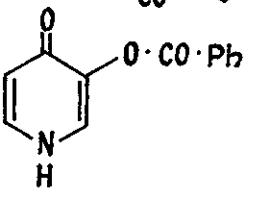
X $n = 2$, R = $OCH_2 \cdot CH_2 \cdot N$



XI



XII



XIII

REFERENCES

1 P.J. Reis, Aust. J. Biol. Sci., 1975, 28, 483.

2 A.F. Bickel and J.P. Wibaut, Recl. Trav. Chim. Pays-Bas, 1946, 65, 65

3 M.P. Hegarty, P.G. Schinckel, and R.D. Court, Aust. J. Agric. Res., 1964, 15, 153.

4 M.P. Hegarty, R.D. Court, and P.M. Thorne, Aust. J. Agric. Res., 1964, 15, 168.

5 R.L.N. Harris, Aust. J. Chem., 1976, 29, 1329.

6 I. Flemming and D. Phillippides, J. Chem. Soc. C, 1970, 2426.

7 Y. Ueno, T. Takaya, and E. Imoto, Bull. Chem. Soc. Jpn, 1964, 37, 864.

Received, 12th July, 1977