A SYNTHESIS OF MIMOSINE

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ABSTRACT

DL-Mimosine has been synthesized by debenzylation and detosylation of the product obtained by condensation of 3-benzyloxy-4-pyrone with β-amino-α-tosylaminopropionic acid. A new method for the isolation of mimosine from *Leucaena glauca* Benth. is described.

The amino acid mimosine (VIII ↔ XV) was first isolated (1) from the sap of *Mimosa pudica* L. Investigation of its structure (1, 2) and of the structure of leucaenol (leucaenine) (3–9), isolated somewhat later (10) from *Leucaena glauca* Benth. (Kao Haole), made it likely that the two compounds were identical (1, 3–7). Direct comparison (11) confirmed this identity[1] and a synthesis of mimosine has been reported (12).

In connection with an investigation of the biosynthesis of mimosine, we required the compound in quantities which could not be conveniently obtained from *M. pudica*. Attempts to repeat the reported synthesis were disappointing. By reaction of 3-methoxy-4-pyridone (III) with α-acetamidoacrylic acid (IV), Adams and Johnson (12) had obtained an uncharacterized adduct, regarded as *O*-methyl-*N*-acetylmimosine (V), which gave mimosine (VIII) in 25% yield on vigorous hydrolysis with hydriodic acid. We were unable to obtain the amino acid by this method. The final product of the reaction was a mixture which, on the basis of paper chromatographic analysis, did not contain mimosine but appeared to consist mainly of 3-hydroxy-4-pyridone (VI) and alanine.

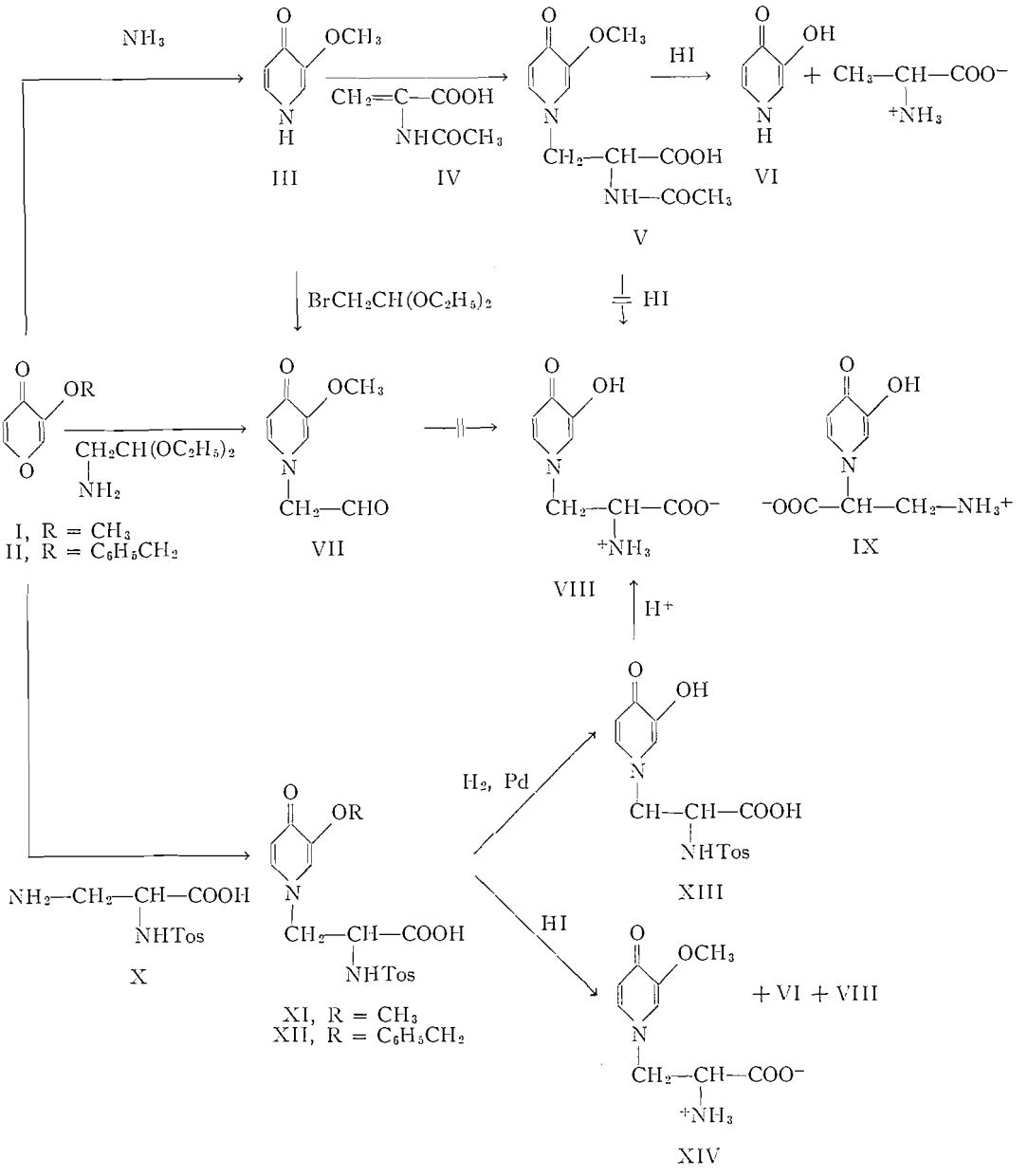
Another approach to synthesis, which had failed, was by the Strecker route. Although condensation of 3-methoxy-4-pyridone (III) with bromoacetaldehyde diethylacetal (13) and condensation of 3-methoxy-4-pyrone (I) with aminoacetaldehyde diethylacetal (14) gave derivatives of the requisite aldehyde (VII), further conversion of these to mimosine proved unsuccessful (13, 15).

The present synthesis was based on the condensation of suitably substituted derivatives of 3-hydroxy-4-pyrone and α,β-diaminopropionic acid. It had been reported earlier that 3-hydroxy-4-pyrone or 3-methoxy-4-pyrone did not react with glycine, α,β-diaminopropionic acid, β-amino-α-bromopropionic acid (15), or with β-amino-α-hydroxypropionic acid (12). Since the crucial step in the pyrone–pyridone conversion is generally regarded as analogous to carbinolamine formation (16), a nonprotonated amine is required as the nucleophilic reactant, and it was likely that the failure of these amino acids to condense was due to their zwitterion structure, in which the amino group is protonated. No attempt had been made to control the ionic state of the reactants (12, 15). Indeed, it was subsequently shown (17) that condensation of glycine with a number of 4-pyrone derivatives takes place only in the presence of an equimolar amount of base.

The desired condensation of a pyrone with α,β-diaminopropionic acid requires the species $NH_2—CH_2—CH(NH_3^+)COO^-$ of the latter. The assignment of pK values (pK_2, 6.69 (α-NH_2); pK_3, 9.50 (β-NH_2)) (18) indicates that in aqueous solutions this species in unobtainable in significant amounts. The species $NH_3^+—CH_2—CH(NH_2)COO^-$,

[1]*Since the name "mimosine" was the first to be coined (1), proof of identity makes the names "leucaenol" (10), "leucenol" (4), and "leucaenine" (5) redundant.*

which overwhelmingly predominates in the isoelectric range of the amino acid, would on condensation with 3-hydroxy-4-pyrone yield an isomer (IX) of mimosine. Condensation of the pyrone with the amino acid at pH > 11, i.e., with the species NH_2—CH_2—$CH(NH_2)COO^-$, would lead to a mixture of mimosine (VIII) and its isomer (IX). For an unequivocal synthesis of mimosine, protection of the α-amino group was therefore required.

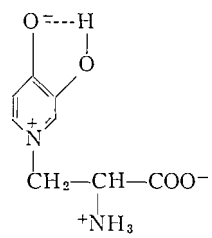


The chosen derivative was DL-β-amino-α-tosylaminopropionic acid (X), which was prepared, according to Rudinger (19), by Hofmann rearrangement of α-*N*-tosylasparagine.

Condensation of the tosylamino acid with 3-methoxy-4-pyrone in the presence of an equimolar quantity of sodium hydroxide gave β-(1,4-dihydro-3-methoxy-4-oxo-1-pyridyl)-*N*-tosylalanine (*O*-methyl-*N*-tosylmimosine) (XI) in 46% yield. Hydrolysis with hydrobromic or hydriodic acid under a variety of conditions gave mimosine (VIII), but was accompanied by considerable *N*-alkyl cleavage. It would appear that whereas mimosine itself is stable towards halogen acids (4, 5), derivatives of its *O*-methyl ether are not; this was presumably the reason why Adams and Johnson obtained a poor yield in their synthesis (12), and why we could not repeat their work. Under conditions where removal of the alanyl side chain from XI was minimal, detosylation did take place, but ether cleavage was incomplete. The product was a mixture of mimosine (VIII) and its *O*-methyl ether (XIV), fractionation of which proved to be tedious.

The difficulty was overcome by a minor variation in the route of synthesis. Condensation of DL-β-amino-α-tosylaminopropionic acid (X) with 3-benzyloxy-4-pyrone (II) gave β-(3-benzyloxy-1,4-dihydro-4-oxo-1-pyridyl)-*N*-tosylalanine (*O*-benzyl-*N*-tosylmimosine) (XII) in 70% yield, which on catalytic debenzylation yielded β-(1,4-dihydro-3-hydroxy-4-oxo-1-pyridyl)-*N*-tosylalanine (*N*-tosylmimosine) (XIII) in yields of 80%. Hydrolysis of the tosyl group by hydrogen bromide in glacial acetic acid gave DL-β-(1,4-dihydro-3-hydroxy-4-oxo-1-pyridyl)-alanine (VIII),[2] melting at 228–230° (decomp.). The melting point and infrared absorption of this synthetic material were very similar to the melting point (11, 12, 20) and infrared absorption[3] (9, 12, 20) of samples of natural mimosine, obtained from *M. pudica* L. and from *L. glauca* Benth. The ultraviolet absorption curves of the synthetic and the natural (4, 9) material were identical.

Synthetic and natural mimosine showed identical dissociation constants (pK_1, 2.1 (COOH); pK_2, 7.2 (NH_3^+); pK_3, 9.2 (OH)). The values for pK_2 and pK_3 are in good agreement with those calculated (7.28 and 9.19 respectively) from reported potentiometric data (5). The dissociation, pK_2, was the only one depressed in the presence of formaldehyde and must therefore be assigned to the α-amino group of mimosine. Its value, unusually low for the α-amino group of an amino acid, corresponds to reported values of the dissociation constants of similar NH_3^+ groups in 1,2-diammonium derivatives (e.g., $NH_3^+CH_2CH(NH_3^+)COO^-$, pK_2, 6.69 (α-NH_3^+) (18); $NH_3^+—CH_2—CH_2—NH_3^+$, pK_1, 6.98 (22)). The factor which lowers the basicity of the NH_2 group in these compounds is the polar effect of the proximal, charged NH_3^+ group. Since the pK_a of the α-amino group of mimosine is of similar magnitude, it is likely that the species XV is an important resonance contributor.



XV

[2]*An attempt to synthesize the* L-*isomer, starting from* L-α-*tosylamino-β-aminopropionic acid, failed, since condensation of this compound with 3-benzyloxy-4-pyrone under our conditions was accompanied by racemization.*

[3]*The observation that the infrared spectrum of a* DL-*amino acid in the solid state (*KBr*) differs from the spectra of the individual* D- *and* L-*isomers has been repeatedly confirmed (e.g., ref. 21).*

Unequivocal proof of identity, and incidentally, confirmation of structure, were obtained by comparison of the nuclear magnetic resonance spectra of synthetic and natural mimosine, recorded on samples dissolved in deuterium oxide containing sodium deuteroxide. A trace of water (H_2O) was present for internal reference. The two spectra were superimposable and showed four signals (chemical shift in parts per million from water): (*a*) a triplet +1.20, +1.12, +1.03 (—CH—); (*b*) a doublet +0.71, +0.63 ($—CH_2—$); (*c*) a doublet −1.57, −1.67 ($C_5—H$); (*d*) a singlet −2.49 ($C_2—H$) superimposed on a doublet −2.52, −2.62 ($C_6—H$). The areas of the signals were in the ratio 1:2:1:2. This spectrum fully accounts for the structure of the amino acid.

Extraction of mimosine from the seeds of *L. glauca* Benth. by reported methods (3–6) requires large volumes of solvent and the removal from the extracts of considerable quantities of protein and polysaccharides (23). Partial hydrolysis of the latter in the course of extraction leads to gummy intractable residues from which the desired product is obtainable only with great difficulty.

A simple but effective method for the separation of mimosine from the seeds of *L. glauca* has now been developed. Finely powdered seeds were dialyzed against distilled water. When the dialyzate was concentrated and allowed to stand overnight, almost pure mimosine crystallized in high yield.

Mimosine was also isolated from the exudate obtained on cutting the stems of *M. pudica*.

In biosynthetic experiments with *M. pudica*, radioactivity from carbon-14 dioxide and DL-3-C^{14}-aspartic acid was incorporated into mimosine. Degradation of the labelled mimosine from the latter experiment is in progress.

EXPERIMENTAL

3-Hydroxy-4-pyrone (Pyromeconic Acid)

3-Hydroxy-4-pyrone, melting at 116–118°, was prepared (24) in 66% yield by pyrolysis of anhydrous meconic acid and was purified by sublimation at 110° and 10^{-3} mm.

3-Methoxy-4-pyrone (I)

3-Methoxy-4-pyrone, melting at 93–95°, was obtained in 84% yield by methylation of pyromeconic acid with diazomethane in ether solution (24). It was purified by distillation at 5×10^{-3} mm and 100–110°.

3-Benzyloxy-4-pyrone (II)

A mixture of 3-hydroxy-4-pyrone (2.24 g, 0.02 mole), potassium iodide (0.30 g, 0.00019 mole), anhydrous potassium carbonate (2.67 g, 0.019 mole), and benzyl chloride (2.70 g, 2.45 ml, 0.022 mole) in dimethylformamide (100 ml) was heated for 8 hours on the steam bath with continuous stirring. The hot mixture was filtered, the residue washed repeatedly with ethanol, the combined filtrates evaporated to dryness, and the residual solid exhaustively extracted with ether. The extract was dried (Na_2SO_4) and concentrated to yield crystals of *3-benzyloxy-4-pyrone* (3.30 g, 81%), melting at 84–85° after recrystallization from ether. (Found: C, 71.0; H, 5.0. $C_{12}H_{10}O_3$ requires: C, 71.3; H, 5.0%.)

L- *and* DL-*β-Amino-α-tosylaminopropionic Acid (X)*

This was prepared according to Rudinger *et al.* (19) from L- and DL-*α-N*-tosylasparagine respectively.

β-(1,4-Dihydro-3-methoxy-4-oxo-1-pyridyl)-N-tosylalanine (O-Methyl-N-tosylmimosine) (XI)

3-Methoxy-4-pyrone (1.39 g, 0.011 mole) in water (10 ml) was added to a solution of DL-β-amino-α-tosylaminopropionic acid (2.58 g, 0.01 mole) in 0.1 *M* sodium hydroxide (100 ml, 0.01 mole). The mixture was heated for 3 hours on the steam bath and then concentrated to a volume of 50 ml, when the pH, originally above pH 11, had dropped to pH 9. The pH was adjusted to pH 7 by dropwise addition of concentrated hydrochloric acid and the solution was allowed to stand at 5° for several hours. Unreacted tosylamino acid (0.31 g, 0.0012 mole) was filtered off and the pH of the filtrate was adjusted to pH 2 with concentrated hydrochloric acid. Crystallization of the product started almost immediately and was complete after 12 hours at 5°, yielding DL-*β-(1,4-dihydro-3-methoxy-4-oxo-1-pyridyl)-N-tosylalanine* (1.51 g, 47%), melting at 200–201° (decomp.) after recrystallization from water. (Found: C, 52.7; H, 5.1; N, 7.6; S, 9.0. $C_{16}H_{18}N_2O_6S$ requires: C, 52.5; H, 5.0; N, 7.7; S, 8.7%.)

Condensation of 3-methoxy-4-pyrone with L-β-amino-α-tosylaminopropionic acid in place of the DL-compound was accompanied by racemization, also yielding optically inactive condensation product.

β-(3-Benzyloxy-1,4-dihydro-4-oxo-1-pyridyl)-N-tosylalanine (O-Benzyl-N-tosylmimosine) (XII)

3-Benzyloxy-4-pyrone (2.24 g, 0.011 mole) in ethanol (15 ml) was mixed with a solution of DL-β-amino-α-tosylaminopropionic acid (2.58 g, 0.01 mole) in 0.1 *M* sodium hydroxide (100 ml, 0.01 mole). The mixture was warmed and shaken until homogeneous, and the solution was heated on the steam bath under reflux for 8 hours. Concentrated hydrochloric acid (3 ml) was added with rapid stirring and the solution allowed to stand at 5° overnight, when *β-(3-benzyloxy-1,4-dihydro-4-oxo-1-pyridyl)-N-tosylalanine* (3.25 g, 74%), melting at 203–205° (decomp.) after recrystallization from ethanol, was obtained. (Found: C, 59.5; H, 5.3; N, 6.3; S, 7.4. $C_{22}H_{22}N_2O_6S$ requires: C, 59.7; H, 5.0; N, 6.3; S, 7.2%.)

β-(1,4-Dihydro-3-hydroxy-4-oxo-1-pyridyl)-N-tosylalanine (N-Tosylmimosine) (XIII)

O-Benzyl-*N*-tosylmimosine (1.00 g, 0.0023 mole) in dimethylformamide (100 ml) was shaken 45 hours at room temperature under hydrogen at 18 p.s.i. in the presence of 5% palladium on charcoal (1.00 g), the catalyst having been prehydrogenated at 25 p.s.i. in ethanol (50 ml) at room temperature for 3 hours. The reaction mixture was heated and filtered and the catalyst exhaustively washed with hot ethanol. The filtrate was evaporated to dryness and the residue crystallized from ethanol, yielding *β-(1,4-dihydro-3-hydroxy-4-oxo-1-pyridyl)-N-tosylalanine* (0.64 g, 80%), melting at 203–205° (decomp.). (Found: C, 51.0; H, 4.8; N, 7.8; S, 9.3. $C_{15}H_{16}N_2O_6S$ requires: C, 51.1; H, 4.6; N, 8.0; S, 9.1%.)

β-(1,4-Dihydro-3-hydroxy-4-oxo-1-pyridyl)-alanine (DL-Mimosine) (VIII)

The tosyl derivative (XIII) (0.40 g) was dissolved in sufficient glacial acetic acid (approximately 25 ml) to give a homogeneous solution. Phenol (0.40 g) was added and the solution was saturated at room temperature with dry hydrogen bromide and left at 60–65° in a stoppered flask. The reaction mixture was repeatedly monitored by ascending paper chromatography (phenol–ethanol–water, 3:1:1) and incubation was continued until the phenolic spot corresponding to *N*-tosylmimosine (R_f 0.78) failed to appear and only that corresponding to mimosine (R_f 0.27) was observed on development of the chromatogram with ferric chloride solution. This generally required 2–3 days but in some runs additional hydrogen bromide was passed into the solution after 3 or 4 days in order to complete hydrolysis. The cooled solution was diluted with dry ether (400 ml) and allowed to stand at 5° until the separation of mimosine hydrobromide was complete. The supernatant liquid was decanted and the residue repeatedly extracted with ether and then dissolved in water. The aqueous solution was basified with concentrated ammonium hydroxide and evaporated to dryness under reduced pressure. The remaining solid was repeatedly moistened with water and evaporated to dryness under reduced pressure to remove excess ammonia, and finally dissolved in hot water, decolorized with charcoal, and allowed to crystallize, yielding DL-mimosine (0.10 g, 45%) melting at 222–225° (decomp.). For analysis a sample was recrystallized from boiling water and the product filtered from the hot solution, to give anhydrous DL-mimosine, melting at 228–230° (decomp.). (Found: C, 48.7; H, 5.4; N, 14.1. Calculated for $C_8H_{10}N_2O_4$: C, 48.5; H, 5.1; N, 14.1%.) Dissociation constants (determined by half-titration of mimosine in 0.04 *M* aqueous solution with 0.10 *M* sodium hydroxide and 0.10 *M* hydrochloric acid, and application of water correction): pK_1, 2.1 (—COOH); pK_2, 7.2 (α-NH_3^+); pK_3, 9.2 (phenolic —OH). Only pK_2 was depressed in the presence of formaldehyde. Ultraviolet absorption (λ_{max}, mμ (log ε)): in water: 283 (410); in 0.07 *M* HCl: 277 (3.87); in 0.07 *M* NaOH: 309 (4.04). Infrared absorption (KBr) (cm^{-1}): 3400 (broad) (OH), 2900 (broad) (NH), 1640 (s) (C=O), 1588 (s) (COO^-), 1490 (s) (NH_3^+).

Mimosine from the Seeds of Leucaena glauca

Coarsely crushed dried seeds were pulverized to a fine powder in a ball mill. The powdered seeds (50 g) were placed into a dialysis bag (length 35 cm, cross section 3 cm) which was then filled with distilled water and completely immersed in distilled water (1 l.). After three changes of external solvent the dialyzate showed only a weak phenolic reaction. Each dialyzate was concentrated at reduced pressure to 25 ml, when almost pure product crystallized. Mimosine (0.61 g, 1.2%), melting at 227–228° (decomp.), after recrystallization from water, was obtained from 50 g of seeds. Dissociation constants: pK_1, 2.1; pK_2, 7.2 (α-NH_3^+); pK_3, 9.2 (OH). Ultraviolet absorption: (λ_{max}, mμ (log ε)): in water: 283 (4.11); in 0.07 *M* HCl: 277 (3.86); in 0.07 *M* NaOH: 309 (4.04). Infrared absorption (KBr) (cm^{-1}): 3400 (broad) (OH), 2850 (broad) (NH), 1640 (s) (C=O), 1590 (s) (COO^-), 1530 (s), 1490 (s) (NH_3^+).

Mimosine from Mimosa pudica (cf. Ref. 1)

The exudate from freshly cut green stems and petioles of *Mimosa* was aspirated into a lambda pipette. A sample (250 μl) was rubbed with ethanol (5 ml), when crude mimosine (12 mg) precipitated, which after treatment with charcoal and recrystallization from water melted at 224–226° (decomp.). A sample of exudate (250 μl) was evaporated to dryness in a vacuum desiccator to give a brown residue (39 mg) which was dissolved in water, decolorized with charcoal, and yielded mimosine, melting at 221–223° (decomp.).

A sample of exudate (250 μl) was dissolved in phthalate buffer (pH 4, 0.2 *M*) (3 ml), and applied to a cation exchange column (Dowex 50-X4) in the hydrogen form. Water eluted a fluorescent material which was not further investigated. Mimosine was displaced with ammonia (0.02 *M*). The eluate was concentrated *in vacuo* and dried in a vacuum desiccator over concentrated sulphuric acid, yielding mimosine (9 mg), melting at 225–226° (decomp.).

Paper Chromatography

Reaction mixtures and plant extracts were monitored by ascending paper chromatography on Whatman No. 1 paper, using phenol–ethanol–water, 3:1:1, as the solvent. Spots were developed with ferric chloride (1%) and/or ninhydrin (3% in acetone). Before development the papers were dried for 1 hour at 80° to ensure complete removal of phenol.

Under these conditions the following R_f values were found: 3-hydroxy-4-pyrone, R_f 0.83; 3-hydroxy-4-pyridone, R_f 0.70; *O*-methylmimosine, R_f 0.61; *N*-tosylmimosine, R_f 0.78; mimosine, R_f 0.27.

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