Synthetic Studies on Peganum harmala Alkaloids

Part I. New derivatives of harmol.

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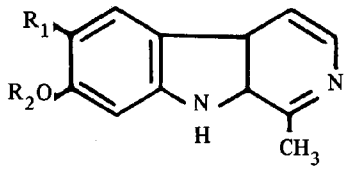
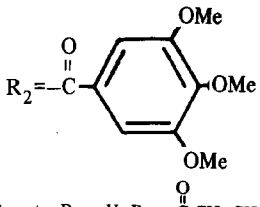
Summary: Structure and activity correlationship studies in harmol have yielded a number of new derivatives; their structures have been established through chemical characterization and spectral data.

Studies in the alkaloidal constituents of *Peganum harmala* started in 1841 and have been invariably based on the extracts of the crushed seeds of the plant[1-8]. During the study on the germination metabolites of the seeds[9] it was discovered that the alkaloids are wholly located in the husk which forms about 50% of the seeds and that the Kernel yields 20% of oil which is of edible quality. The economic viability of the harmal seeds as a new source of edible oil would considerably depend on the appropriate utilization of the alkaloids available from the husk in about 7% yield. Intensive studies have been undertaken therefore on the chemistry of the alkaloids harmine,[10] harmaline[1] and their derivatives. In an earlier communication, N. Afza and S. Siddiqui have reported a number of synthetic analogues of tetrahydroharmine through an extension of von Braun cyanogen bromide reaction[11]. This emphasis has now been extended to the derivatives of harmol[12], available in good yield through the demethylation of harmine.

Following the procedures described in the experimental, the following derivatives were obtained and their structures established through chemical and spectral studies. They were further characterized through their various salts.



1. Harmol, R_1 = H; R_2 = H
2. 6-nitroharmol, R_1 = NO_2; R_2 = H
3. 6-aminoharmol, R_1 = NH_2; R_2 = H
4. Harmol-3,4,5-trimethoxybenzoate, R_1 = H;



5. Harmol crotonate, R_1 = H; R_2 = $\overset{O}{\overset{\|}{C}}$-CH=CH-$CH_3$
6. O-allylharmol, R_1 = H; R_2 = CH_2-CH=CH_2
7. O-2,5,8-nonatrienylharmol, R_1 = H; R_2 = CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH_2.

Nitration of harmol had to be carried out under highly critical conditions (yield 85%). Its reduction with zinc and hydrochloric acid yielded aminoharmol (yield 51%). The positions of the nitro and amino groups were ascertained to be at C-6 by nmr spectroscopy. In the aromatic region, there is a peak at δ 7.1 due to H-8 which shows a small splitting of 0.2 Hz due to para coupling with H-5. The signal of H-5 is at δ 8.72 with the same small coupling constant. H-3 and H-4 show a typical AB pattern; there is a complex multiplet of peaks at about δ 8.05 due to two protons with a coupling constant of 6 Hz.

Reaction of crotonoyl chloride with an alcoholic suspension of harmol at room temperature, afforded harmol crotonoate in 95% yield. However, esterification of harmol with 3,4,5-trimethoxybenzoylchloride presented considerable difficulties and the 3,4,5-trimethoxy benzoate could be obtained only in 25% yield, upon fusion of the reactants according to the procedure described in the experimental.

The action of allyl bromide on harmol furnished a

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binary mixture of products from which the O-allyl derivative was obtained as yellowish orange viscous liquid (yield 50%). Another product of the reaction obtained in 10% yield was 0-2,5,8-nonatrienylharmol. The latter is probably formed through the trimerization of allyl bromide, which then reacts with harmol to furnish the nonatrienyl derivative. It may be noted that the yield of this product increased to 68% when the period of heating the reactants was extended from 2 to 4 hours.

Experimental

Nitration of harmol:

To a solution of 1 g harmol in 10 c.c. glacial acetic acid was gradually added 10 c.c. of nitric acid (d.1.4). The temperature was kept below 30°C with occasional cooling. The initial yellow colour of the reaction mixture thereby turned to deep red. Within about a minute, a drop taken out did not show the characteristic violet fluorescence of harmol in UV light. The reaction was quenched at this stage by pouring the mixture into crushed ice, when reddish orange crystals separated out. These were filtered, repeatedly washed with water and dried on a porous plate. The nitrate salts of the nitro derivative was dissolved in hot water, and treated with 10% ammonium hydroxide. The pale yellow crystalline precipitate was washed with water, and dried on the porous plate. The base was dissolved in methanol with the addition of a little benzene and kept in the cold, when 6-nitroharmol precipitated as deep yellow slender needles in about 85% yield. On recrystallization from methanolic benzene, it melted at 350°C. (decomp.) and analyzed for $C_{12}H_9N_3O_3$ (Found: C, 59.38; H, 3.52; N, 17.22%). Calcd. for $C_{12}H_9N_3O_3$ C, 59.26; H, 3.70; N, 17.28%). The IR spectrum of the base in KBr shows peaks for NH/OH (3200-34 cm^{-1}); nitro group (1555 cm^{-1}) and aromatic ring (1628 cm^{-1}). The mass spectrum includes molecular ion peak m/e 243 and other diagnostic peaks m/e 213 $(M\text{-}NO)^+$ and m/e 197 $(M\text{-}NO_2)^+$. The proton NMR spectrum in deuterated trifluoroacetic acid shows that the nitro group is present at C-6.

Salts of 6-nitroharmol:

The hydrochloride, hydrobromide, hydroiodide, nitrite, nitrate and picrate salts of the nitro base were prepared by treating dilute acetic acid solution of the base with the corresponding alkali salts and picric acid respectively, followed by crystallization from methanol. The chloroplatinate, prepared by adding 3% solution of platannic chloride to the aqueous solution of the hydrochloride of the base, formed a reddish orange powder. None of these salts melted upto 360°C.

Reduction of 6-nitroharmol:

Finely powdered 6-nitroharmol (0.5 g) was taken in 8 c.c. of 20% hydrochloric acid and small portions of zinc dust added with slight heating on the water bath. The initial suspension thereby went into solution. In about half an hour, the yellowish solution became nearly colourless. It was filtered, washed with water and the filtrate was treated with ammonia after the addition of saturated solution of ammonium chloride. The liberated base was extracted out with ethyl acetate. The residue left on removal of the solvent after washing and drying was taken up in dilute acetic acid and treated with potassium iodide. The light yellowish precipitate of the hydroiodide of the amino base crystallized from methanol in aggregates of slender needles which melted at 335°C. On treatment of the hydroiodide with dilute ammonia and crystallization of the liberated base from methanol with the addition of little benzene, 6-aminolharmol was obtained in the form of colourless needles which melted at 350°C. (yield 50%) and analysed for $C_{12}H_{11}N_3O$ (Found: C, 67.67; H, 5.20; N, 19.63%. Calcd. for $C_{12}H_{11}N_3O$: C, 67.60; H, 6.16; N, 19.17%). The mass spectrum shows molecular ion peak m/e 213.

Salts of 6-aminoharmol:

The salts of 6-aminoharmol were prepared in exactly the same manner as described for 6-nitroharmol, -dihydrochloride m.p. 300°C. (decomp.); dihydrobromide m.p. 335°C. (decomp.); -dihydroiodide m.p. 312°C. (decomp.) and dipicrate mp. 222°C.

Harmol crotonoate:

Freshly distilled crotonoyl chloride (2 c.c.) was added to a suspension of harmol (0.5 g) in 10 c.c. of alcohol. On continued stirring for about two hours at room temperature, a thick crop of colourless crystallizate settled out which filtered after cooling in crushed

ice, and washed with a little cold water. The hydrochloride of the base thus obtained was taken up in hot water and treated with ammonia, whereby crotonoyl harmol separated out as a crystallizate. On recrystallization from methanol it formed colourless needles which melted at 178-9°C. and analysed for $C_{16}H_{14}N_2O_2$ (Found: C, 72.26; H, 5.19; N, 10.46%. Calcd. for harmol crotonoate $C_{16}H_{14}N_2O_2$: C, 72.18; H, 5.26; N, 10.52%). The IR spectrum in KBr shows peaks at 3400 cm^{-1} (indolic NH), 1730 cm^{-1} (C=O), 1628 cm^{-1} (aromatic ring), 1615 cm^{-1} (conjugated C=C) and multiple bands between 1160-1300 cm^{-1} (O-CO stretching of α, β-unsaturated esters). The mass spectrum gives molecular ion peak m/e 226 and other diagnostic peaks m/e 197 $(M\text{-}Co.CH{=}CH\text{-}CH_3)^+$; and m/e 180 $(M\text{-}HOOC.\ CH{=}CH\text{-}CH_3)^+$. The strong peaks m/e 86 and 69 established the presence of crotonoic acid and crotonoyl ion respectively. On the other hand the $^1H_1$ nmr spectrum shows in addition to the normal peaks of harmol, a one proton octet downfield at δ 7.1 for conjugated olefinic proton, while the other olefinic proton appears as a doublet at δ 5.65. The three proton doublet at δ 1.98 can be assigned to the methyl group attached directly to C=C.

Salts of harmol crotonoate:

Prepared according to the procedure described for the salts of 6-nitroharmol, -hydrochloride m.p. 262-3°C; -hydrobromide m.p. 254°C; -hydroiodide m.p. 275-6°C and picrate m.p. 204-5°C.

Harmol-3,4,5-trimethoxybenzoate:

Harmol, 0.5 g., was fused with 1 g. 3,4,5-trimethoxy benzoyl chloride, freshly prepared by the action of thionyl chloride on 3,4,5-trimethoxybenzoic acid. The fused mass was heated with mechanical stirring on a hot plate till the evolution of hydrogen chloride ceased. Absolute alcohol (10 c.c.) was added to the reaction mixture and kept overnight in the ice chest. The colourless crystalline mass that congealed was subjected to three cornered fractional crystallization from absolute alcohol when harmol 3,4,5-trimethoxybenzoate was obtained from the top fractions as fine colourless needles. These on repeated crystallizations from the same solvent finally melted at 179-80°C and analyzed for $C_{22}H_{20}N_2O_5$ (Found: C, 67.41; H, 5.22; N, 7.08% Calcd. for $C_{22}H_{20}N_2O_5$: C, 67.34; H, 5.10; N, 7.14%). The IR spectrum in kBr shows indolic NH (3400 cm^{-1}), C=O stretching (1730 cm^{-1}), aromatic ring (1628 cm^{-1}) and O-CO stretching of α,β-unsaturated ester (1660-1300 cm^{-1}). The mass spectrum has molecular ion peak me 392, followed by diagnostic peaks m/e 197 (M-trimethoxy benzoyl)$^+$ and 180 (M-trimetoxy benzoic acid)$^+$. The intense peaks m/e 212 and 195 establish the presence of 3,4,5-trimethoxybenzoic acid and 3,4,5-trimethoxybenzoyl ion, respectively. The proton NMR spectrum also indicates the presence of the trimethoxybenzoyl moiety. In addition to the normal peaks of harmol, it shows a 6H singlet at δ 3.82 and a 3H singlet at δ 3.89 which can be assigned to the aromatic methoxyl groups. Moreover, the integration curve in the aromatic region indicates the presence of two additional protons due to the trimethoxy benzoyl group.

Salts of 3,4,5-trimethoxy benzoate:

The following salts were prepared according to the procedure noted in the preceding account: -hyrdochloride m.p. 251-2°C; -hydrobromide m.p. 247°C; -hydroiodide m.p. 204°C and picrate m.p. 239-40°C.

O-allylharmol:

Harmol 0.5g was taken up in 10 c.c. of 5% alcoholic sodium hydroxide, and 2 c.c. of freshly distilled allyl bromide was added on to it. The mixture was refluxed for two hours on water bath, poured into crushed ice, and exhaustively extracted out with ethyl acetate. The ethyl acetate extract was repeatedly washed with water, dried filtered, and left for slow evaporation at room temperature. The filtrate from a small quantity of crystallizate that separated out was freed of the solvent. The residue was taken up in dilute acetic acid and treated with KI. The crystalline hydroidoide of the base thereby obtained was repeatedly recrystallized from methanol, when it finally melted at 37°C and analysed for $C_{15}H_{14}N_2O$. HI (Found: C, 49.29; H, 4.17; N, 7.72; I, 34.43%. Calcd. for $C_{15}H_{14}N_2O$.HI: C, 49.18; H, 4.09; N, 7.65; I, 34.69%). The allyl base liberated from the hydroiodide formed a yellowish orange viscous liquid and gave a single spot on T.L.C.

The yellowish crystallizate referred in the above working was subjected to repeated crystallization from methanol, when it furnished lemon yellow prismatic rods which melted at 218°C and analysed for $C_{21}H_{22}N_2O$.

(Found: C, 79.30; H, 6.86; N, 8.89%. Calcd. for 0-2, 5,8-nonatrienyl harmol $C_{21}H_{22}N_2O$: C, 79.24; H, 6.91; N, 8.80%).

Characterization of O-allylharmol:

The IR spectrum of the allyl base in KBr shows indolic NH(3400 cm^{-1}) and C=C (1640 cm^{-1}). The mass spectrum includes molecular ion peak m/e 238 in agreement with the molecular formula $C_{15}H_{14}N_2O$ for O-allylharmol. The presence of O-allyl group is demonstrated by diagnostic fragments m/e 198 and 197 which result from the loss of allyl group with or without transfer of one hydrogen atom to the indole moiety. Further confirmation was provided by $^1H_1$ nmr spectrum. Besides the normal peaks of harmol, it includes a two proton distorted doublet at δ 6.00 for the olefinic methine proton in the grouping CH_2 =CH – CH_2-O. The two protons of the olefinic methylene group come as multiplets separately at δ 5.28 and δ 5.13, respectively, because they are not chemical equivalent.

Characterization of O-2,5,8-nonatrienylharmol:

The IR spectrum of the base in KBr exhibits indolic NH (3400 cm^{-1}) and non conjugated C=C at 1640 cm^{-1}. The mass spectrum shows molecular ion peak m/e 318 in agreement with the molecular formula $C_{21}H_{22}N_2O$. The peak m/e 278 represents the loss of the allyl group with hydrogen transfer to the indole moiety, and peaks m/e 198 and 197 arise from the complete loss of the long alkyl chain with or without H transfer to the indole moiety. The proton nmr spectrum has a very complex pattern in the olefinic region, but the integration curve accounts for seven protons in accordance with the proposed structure of the product.

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