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FURTHER STUDIES IN THE AJMALINE SERIES

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Abstract. The preparation of a number of new derivatives of ajmaline and isoajmaline with substitution in the aromatic ring of the two bases are described. NMR studies revealed that the electrophilic substitution in ajmaline takes place at C-10.

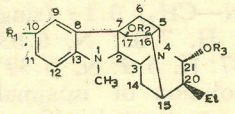
In earlier communications of the series,[1,2] it was observed that the introduction of certain electronegative groups like Br and NO_2 in the aromatic ring of the ajmaline molecule (I) has a potentiating effect on the antiarrhythmic action of the base. It has been found[3] that the functional refractory period of the guinea pig's left atrium is prolonged by 24.05% by bromoajmaline and 23.3% by nitroajmaline as against 10.16% by ajmaline and 6.33% by quinidine sulphate at a final bath concentration of 10^{-6} g/ml.

The high antiarrhythmic activity of bromoajmaline led us to evolve a better method for the preparation of the derivative. It was found that ajmaline can be smoothly brominated by stirring its methanolic solution with cupric bromide and that on working up the reaction mixture after basifying with ammonia monobromoajmaline (II) is obtained in 64.5% yield* as against 30% reported by Robinson *et al.*[4]

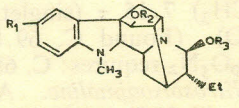
In continuation of the studies on the structure activity relationship in the ajmaline series a number of derivatives have been prepared with the introduction of various groups in the aromatic ring of ajmaline. Formylajmaline (III) was obtained by Vilsmeir formylation of diacetylajmaline yielding, 10-formyl-17, 21-diacetylajmaline (IV), m.p. 236°C (yield 70%) and its mild alkaline hydrolysis afforded 10-formylajmaline, m.p. 270°C. 10-Formyl-17,21-diacetylajmaline could be reduced with lithium aluminium hydride to 10-hydroxymethylajmaline (V), the acetyl groups being removed during the process. Friedel–Crafts acylation of diacetylajmaline with acetyl chloride in the presence of anhydrous aluminium chloride afforded a pale amorphous powder which could not be induced to crystallisation. On mild alkaline hydrolysis, however, it gave 10-acetylajmaline (VI) as light yellow rectangular plates, m.p. 236–37°C, yield 51%.

The present study has also been extended to isoajmaline (IX). Following new derivatives of this base have been prepared by employing the procedures described in the experimental:

10-nitro-17,21-di-*O*-acetylisoajmaline (X), 10-formyl-17,21-di-*O*-acetylisoajmaline (XI), 10-nitrosoisoajmaline (XII),10-amino-17,21-di-*O*-acetylajmaline (XIII).



I to VIII



IX to XIII

*Yield calculated on theoretical basis.

where I, R_I, R_2, R_3=H, II, R_I=Br; R_2, R_3=H; III; R_I=CHO; R_2, R_3 = H; IV, R_I = CHO; R_2, R_3 = $COCH_3$; V, R_I = CH_2OH, R_2, R_3 = H; VI, R_I = $COCH_3$; R_2, R_3=H; VII, R_I, R_2 = H; R_3 = $COCH_3$; VIII; R_I, R_3 = H; R_2 = $COCH_3$; IX, R_I, R_2, R_3 = H; X, R_I = NO_2; R_2, R_3 = $COCH_3$; XI, R_I = CHO, R_2, R_3 = $COCH_3$; XII, R_I = NO; R_2, R_3=H; XIII, R_I = NH_2; R_2, R_3 = $COCH_3$.

Since nitrosation of ajmaline in dilute acetic acid medium yields *N*-nitroso derivative,[5] acetylation of the base in the same medium with acetic anhydride was attempted with the aim of obtaining the hitherto undescribed *N*-acetylajmaline. However 21-*O*-acetylajmaline (VII) was obtained in 70% yield, and this constituted a better method of preparation than that described by Bartlett *et al.*,[6] which is cumbersome and also gives lower yields (52%).

When a solution of di-*O*-acetylajmaline in methanol was allowed to stand at room temperature, it slowly changed into another compound which on separation from the unchanged base by thin layer chromatography could be identified as 17-acetylajmaline (VIII). As expected, the 21-*O*-acetyl group undergoes methanolysis at a faster rate than the 17-*O*-acetyl group, but if the reaction is allowed to proceed for a longer period both the acetyl groups are removed and ajmaline is obtained. 17-Acetylajmaline exists in two polymorphic forms; when crystallised from ether it melted at 150°C but crystallisation from ethanol afforded crystals melting at 214–215°C (lit.[7] m.p. 213–214°C).

The electrophilic substitution in the aromatic ring of ajmaline may be expected to take place at positions 10 and 12. NMR spectral studies of the substitution products indicated that the groups have entered at position 10. For example, the aromatic region of the NMR spectrum of nitroajmaline shows a doublet at 3.01 τ (J 9 c/s) and may be assigned to H-12 which has a higher electron density, shows ortho coupling with H-11, and a small para coupling with H-9 (J 0.6 c/s) visible under high resolution only. The signal H-11 is a distorted quartet centred at 1.65 τ which is shifted downfield, due to the electron withdrawing nature of the nitro group at C-10. It shows ortho coupling with H-12 (J 9 c/s) and meta coupling with H-9 (J 2.50 c/s). The H-9 signal is again a doublet at 1.5 τ showing meta coupling with H-11 (J 2.50 c/s). All other substitution products show similar NMR pattern in the aromatic region.

Experimental

All m.ps were taken in glass capillary tubes and are uncorrected. IR spectra were recorded on Perkin–Elmer instrument. NMR spectra were taken at 60 mc/s in $CDCl_3$ unless otherwise stated.

10-*Acetylajmaline*. A solution of diacetylajmaline (1.00 g, 0.0024 mole) in CS_2 (10 ml) was added to a mixture of $AlCl_3$ (2.0 g, 0.015 mole) and acetyl chloride (10 ml) under ice cooling. The contents of the flask were heated under reflux on the water-bath for 2.5 hr whereby the initially viscous yellow lower layer turned into a brown semisolid mass. The reaction mixture was treated with HCl (dil) in the cold and CS_2 was removed by extraction with ethyl acetate. The aqueous acidic solution was basified with ammonia and extracted exhaustively with ethyl acetate from the insoluble aluminium hydroxide. The residue obtained on removal of ethyl acetate was treated with a 5% sodium hydroxide solution in dilute methanol (10 ml) for 20 min in order to saponify *O*-acetyl groups. The solution was then acidified with dil HCl, methanol evaporated under reduced pressure, residue taken up in water, basified with ammonia and the resulting precipitate extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried (Na_2SO_4) and concentrated whereby 10-acetylajmaline crystallised in rectangular plates (0.51 g), m.p. 236–37°C. Its IR spectrum showed peaks at 1640 cm^{-1} (aromatic ketone) and 3410 cm^{-1} (OH). (Found: C, 71.72; H, 7.69; N, 7.30%. $C_{22}H_{28}N_2O_3$ requires: C, 71.71; H, 7.66; N, 7.60%).

10-*Formyl*-17,21-*di*-O-*acetylajmaline*. A mixture of phosphorus oxychloride (0.42 ml, 0.0027 mole) and *N*-methylformanilide (0.6 ml, 0.0048 mole) was allowed to stand in the ice-bath till the formation of the complex was complete (ca. 30 min). A solution of diacetylajmaline (1.0 g, 0.0024 mole) in *O*-dichlorobenzene (6 ml) was added to this complex and the mixture heated in the water-bath at 70–80°C for 1 hr. The reaction mixture was then cooled, treated with ice water and shaken with ethyl acetate. The ethyl acetate was extracted with 20% acetic acid solution and the acidic extract was combined with the aqueous layer. It was then basified with dilute ammonia in the cold and extracted with ethyl acetate. The ethyl acetate extract was washed, dried (Na_2SO_4) and concentrated whereby 10-formyl-17,21-di-*O*-acetylajmaline crystallised as colourless prisms, m.p. 236°C (yield 0.70 g). It showed IR absorptions at 1660 cm^{-1} (conjugated aldehyde) and 1730 cm^{-1} (*O*-acetyl) but no OH absorption. NMR peaks at 7.84 τ, 7.69τ (two singlet $OCOCH_3$), 7.04 τ (singlet $N—CH_3$), 0.1 τ (singlet—CHO). (Found C, 68.70; H, 6.71; N, 6.17%. $C_{25}H_{30}O_5N_2$ requires: C, 68.47; H, 6.9; N, 6.39%).

10-*Formylajmaline*. 10-Formyl-17,21-di-*O*-acetylajmaline (50 mg) was dissolved in 2% methanolic KOH solution (1 ml) and kept at room temperature. After about 5 min colourless crystals separated which were filtered, washed well with water and dilute methanol. On recrystallisation from methanol 10-formylajmaline was obtained as colourless rectangular prismatic rods, m.p. 270°C, IR peaks at 1660 cm^{-1} (—CHO) and 3390 cm^{-1} (OH). (Found C, 70.84; H, 7.43; N, 7.75%. $C_{21}H_{26}N_2O_3$ requires: C, 71.16; H, 7.39; N, 7.9%).

10-*Hydroxymethylajmaline*. A solution of 10-formyl-17,21-di-*O*-acetylajmaline (1.0 g) in dry tetrahydrofuran (10 ml) was gradually treated with lithium aluminium hydride (0.2 g) and the mixture was refluxed for 6 hr. The excess of hydride was decomposed with ice, diluted with water and extracted with ethyl acetate. The organic layer was washed with little water and evaporated under reduced pressure. The residue was extracted with hot ethyl acetate. On concentration of the latter a colourless precipitate separated which crystallise on rubbing and keeping in the cold. It was filtered and recrystallised from a large quantity of acetone in which it is sparingly soluble, clusters of prismatic rods (yield 0.5 g) m.p. 225°C. (Found C, 70.8; H, 7.9; N, 7.48%. $C_{21}H_{28}N_2O_3$ requires: C, 70.76; H, 7.92; N, 7.86%). The compound showed no carbonyl stretching vibration in the IR spectrum.

10-*Nitro*-17,21-*di*-O-*acetylisoajmaline*. Di-*O*-acetyl isoajmaline (1 g) was dissolved in glacial acetic acid (15 ml) and treated drop by drop with a 1:1 (v/v) mixture of nitric acid (d 1.40) and glacial acetic acid under cooling. When the addition of about 0.8 ml nitrating mixture was complete, the colour of the reaction mixture slowly changed to dark green. It was poured over crushed ice at this stage and basified with cold 20% aqueous ammonia. The resulting precipitate was extracted out with ethyl acetate, the latter washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was crystallised from alcohol—yellow rectangular plates, m.p. 245–47°C (yield 0.5 g), IR absorption bands, 1740 cm^{-1} (*O*-acetyl) and 1500 cm^{-1} (NO_2). NMR peaks at 7.85 τ (singlet $OCOCH_3$), 7.75 τ (singlet $OCOCH_3$), 7.15 τ (singlet $N—CH_3$). (Found C, 63.29; H, 6.37; N, 9.23%. $C_{24}H_{29}O_6N_3$ requires: C, 63.28; H, 6.42; N, 9.32%).

10-*Amino*-17,21-di-O-*acetylisoajmaline*. 10-Nitro 17,21-di-*O*-acetylisoajmaline (1 g) was dissolved in 20% hydrochloric acid (5 ml), a pinch of iron dust added and the mixture shaken with slight warming on the water-bath till the solution became colourless. It was filtered from the unreacted iron, basified with aqueous ammonia and extracted with ethyl acetate. The latter was washed with water, dried (Na_2SO_4), concentrated and treated with a little ether whereby 10-amino-17,21-di-*O*-acetylisoajmaline was obtained as microcrystalline powder, it did not melt up to 300°C, IR 1720 cm^{-1} (*O*-acetyl). (Found N, 9.90%. $C_{24}H_{31}O_4N_3$ requires: N, 9.88%).

10-*Formyl*-17,21-*di*-O-*acetylisoajmaline*. This compound was prepared exactly as described for 10-formyl-17,21-di-*O*-acetylajmaline, colourless hexagonal plates from ethyl acetate, m.p. 234–35°C (yield 59%), IR peaks at 1730 cm^{-1} (*O*-acetyl) 1680 cm^{-1} (conj. CHO), NMR peaks at 7.9 τ (singlet $OCOCH_3$) 7.7 τ (singlet, $OCOCH_3$) 7.15 τ (singlet, $N—CH_3$) 0.1 τ (singlet, —CHO). (Found C, 69.83; H, 7.02; N, 6.63%. $C_{25}H_{30}O_5N_2$ requires: C, 68.47; H, 6.9; N, 6.39%).

10-*Nitrosoisoajmaline*. A solution of isoajmaline (1 g) in dil HCl (N/30, 50 ml) was treated in the cold with an aqueous solution of $NaNO_3$ (0.36 g) and then with 15 ml of 1N HCl. The resulting blood-red

solution slowly turned into green. It was basified with aqueous ammonia and extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried (Na_2SO_4) and concentrated whereby 10-nitrosoisoajmaline was obtained as green microcrystalline powder. After recrystallisation from ethyl acetate, it did not melt up to 300°C and showed IR bands at 3400 cm^{-1} (OH), 1500 cm^{-1} (NO). (Found C, 67.64; H, 7.30; N, 10.31%. $C_{20}H_{25}N_3O_3$ requires: C, 67.58; H, 7.09; N, 11.82%).

Bromination of Ajmaline with Cupric Bromide. A solution of ajmaline (0.5 g) in methanol (20 ml) was acidified with a few drops of 50% acetic acid and treated with a solution of $CuBr_2$ (0.5 g) in methanol. The reaction mixture was stirred at room temperature for 3 hr, solvent distilled off on the water-bath, the residue taken up in water and excess of ammonia was added so that all copper salt, remained in solution. The mixture was shaken with ethyl acetate, the latter was washed, dried (Na_2SO_4) and evaporated. The residue was crystallised from methanol, yielding colourless prismatic rods, m.p. 182°C (yield 0.40 g), and was identified as bromoajmaline (lit.[4] m.p. 182°C) by taking mixed melting point with an authentic sample prepared previously. The IR spectra of the two samples were also superimposable.

21-O-*Acetylajmaline.* A solution of ajmaline (1 g) in 10% acetic acid (5 ml) was gradually treated with acetic anhydride (4 ml) with continuous vigorous stirring at room temperature. Ice and water was added and shaken till all acetic anhydride was decomposed, the solution basified with ammonia and extracted with ethyl acetate. The ethyl acetate layer was washed, dried (Na_2SO_4), concentrated whereby the acetylated product was obtained as colourless long thin needles, m.p. 190–192°C (yield 0.7 g). It was identified as 21-*O*-acetylajmaline by taking mixed m.p. and comparison of IR spectra with an authentic sample.

17-O-*Acetylajmaline.* Diacetylajmaline (0.5 g) was dissolved in methanol (5 ml) and kept at room temperature for 24 hr TLC on silica gel PF 254 (Merck) plates (developing solvent, chloroform–methanol, 90:10) showed that about half of the diacetylajmaline (R 0.7) has changed into a slower moving compound (R 0.47). The mixture was separated on two silica gel 254 (Merck) plates (20×20 cm) containing 25 g each of the adsorbant. The lower band on extraction with chloroform and evaporation of solvent yielded a colourless residue which crystallised from ether as colourless rods, m.p. 150–52°C. The m.p. was raised to 212–14°C on recrystallisation from alcohol. The latter polymorph showed no depression in melting point on admixture with an authentic sample of 17-*O*-acetyajmaline prepared as described by Bite *et al.*[7]

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