

SHORT COMMUNICATION

**ANTISPASMODIC ACTIVITY OF THE TERTIARY BASE OF
DAUCUS CAROTA, LINN. SEEDS**

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Summary : A nitrogen containing base responding to Mayer's test and Dragendorff's reagent for tertiary bases has been isolated from the seeds of *Daucus carota* Linn. The effects of the base as its bromide have been studied on smooth muscles of ileum, uterus, blood vessels and trachea of different species of animals. The tertiary base has been found to have papaverine like nonspecific smooth muscle relaxant and spasmolytic activity, but its activity was found to be about one-tenth of that of papaverine.

Key words: *Daucus carota*, Linn. tertiary base papaverine like activity

INTRODUCTION

The water soluble fraction of the alcoholic extract of the seeds of *Daucus carota* was reported to have cholinergic, smooth muscle relaxant and cardiotoxic actions (3). The cholinergic activity was found to be due to the presence of a quaternary base identified as choline (4). Chemical analysis of the alcoholic extract also showed the presence of a tertiary base as evidenced from Mayer's and Dragendorff's tests, the pharmacological actions of which are reported in this communication.

MATERIALS AND METHODS

Chemical

The seeds from recent crop were obtained locally and were sundried till their weight became constant. The powdered seeds, processed in lots of 2 kg each, were defatted with petroleum ether (60-80°C) and then extracted with 90% ethanol. The alcoholic extract was evaporated on a water bath and the residue (48 g) was treated with 5% aqueous HCl solution to extract as completely as possible the bases as their salts. The fraction which dissolved in 5% HCl gave positive tests with Mayer's reagent. Free bases were then regenerated by making it alkaline with ammonia solution and the total liberated tertiary bases were repeatedly extracted with small portions of chloroform. The pooled chloroform extract was washed with distilled water to remove water soluble pigments, and then dehydrated by treatment with potassium sulphate (anhydrous). The chloroform extract was next

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evaporated over water bath to yield a brown viscous residue (270 mg, which was dissolved in methyl alcohol, treated with 1 g activated charcoal, filtered and evaporated on water bath. The alcohol-free residue (267 mg) was dissolved in chloroform and adsorbed on 5 g Alumina (Brockmann), heated on water-bath to remove chloroform completely and this was packed on a column of Alumina (Brockmann) (20 g). Elution was carried out with petroleum ether, petroleum ether-chloroform mixtures and with chloroform. Petroleum ether-chloroform (1:1 and 1:4) and chloroform eluate showed identical spots on T.L.C. plates (Silica gel G plate with chloroform: Methanol 1:1 as solvent and Dragendorff's reagent as spotting agent). These fractions were mixed and evaporated to yield amber coloured amorphous residue (198 mg) which could not be crystallised. The chromatogram of the material on silicic acid impregnated paper (Whatman no. 3 paper immersed for 5 min in a 1% aqueous solution of sodium silicate) using chloroform: methanol (1:1) as developing agent and Rhodamine VI G as spotting agent showed one blue spot under U.V. light, indicating the presence of phosphatidic acid derived from phospholipoid. The chromatogram on buffered (citric acid 0.1 M: disodium hydrogen phosphate 0.2 M in ratio of 279: 121, pH 3.5) paper using organic phase of butylacetate: butanol: water (25:10:10) mixture as solvent and bromophenol blue (1% Sol) as spotting agent showed two blue spots with close Rf values indicating the presence of two basic compounds. Repeated chromatographic separation of this product (192 mg) through Alumina (Brockmann) and then through silica gel columns yielded two single spot materials, both being basic components (20 mg and 171.6 mg, respectively) but resinous and noncrystallisable. The major fraction (171.6 mg) was dissolved in absolute alcohol and Br₂ solution added drop by drop till white flakes appeared. The flask was kept aside overnight securely stoppered for crystallisation and then the crystals were separated. The crystals were recrystallised from absolute alcohol and 14 mg of crystalline compound (m.p. 135°-38°) was obtained. Thin layer chromatography of bromide salt on silica plates with chloroform : ethanol (3:7) as developing solvent and iodine vapour as spotting agent, showed a single elongated yellow spot with Rf 0.423. The yield of this crystalline, highly purified tertiary base bromide was 0.7 mg/100 g of seeds and is mentioned hereafter as DC. The weights of various fractions mentioned in extraction procedure above represent average values of various lots processed. The fraction DC was used for pharmacological studies.

Pharmacological

The effects of aqueous solution of DC were studied on the following preparations :—

1. *Isolated smooth muscles* : Following experiments were conducted :—

Isolated ileum : A 2 cm piece of the ileum of a fasted rabbit, rat or guinea pig was mounted in Tyrode's solution (NaCl 8.0, KCl 0.2, CaCl₂ 0.2, NaHCO₃ 1.0, NaH₂PO₄ 0.05, MgCl₂ 0.01, glucose 1.0 g/l) in an 5 ml isolated organ bath maintained at 37°-38°C. in

which oxygen was constantly bubbled. The qualitative effect of DC was observed mainly in rabbit ileum ($n = 5$) and also in rat ileum ($n = 3$). The quantitative estimation of spasmolytic ED_{50} of DC and its parallel comparison with that of papaverine was conducted on guinea pig ileum by bracketing-dose assay method, using acetylcholine, histamine and barium chloride as spasmogens ($n = 5$ for each spasmogen).

Isolated uterus : Spontaneously contracting nongravid uteri from oestrinised (stilbo-esterol 0.25 mg/kg sc 24 hr before) female albino rats were mounted in 5 ml isolated organ baths as above, but using Dale's solution (NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5, $MgCl_2$ 0.005, glucose 1.0 g/l). In addition to studying the effect of DC *per se* ($n = 5$) its antispasmodic activity against oxytocin, acetylcholine and 5-HT was also qualitatively compared with that of papaverine in 4 experiments.

Tracheal muscle : The effect of DC was observed in 4 experiments on dog's tracheal muscle mounted in isolated organ bath as above using Krebs solution (NaCl 6.9, KCl 0.35, $CaCl_2$ 0.278, $NaHCO_3$ 2.1, KH_2PO_4 0.162, $MgSO_4$ 0.294, glucose 1.0 g/l).

The effects of spasmogens were recorded for 1 min each except 2 min in uterus, and the antispasmodic drugs were allowed to act for 3 min before repeating spasmogens.

II. Perfused blood vessels of frog : The effects of DC and papaverine against barium chloride (10 mg/100 g body wt) induced vasoconstriction of frog's perfused systemic blood vessels were compared in four experiments. The effects of drugs on the perfusion pressure were recorded as described earlier (2).

RESULTS

1. Isolated ileum : DC in concentrations of 25 $\mu g/ml$ and above produced dose related decrease in tone and/or decrease in spontaneous motility of ileum of rabbit and albino rat. The response to 0.25 mg/ml of DC was found to be nearly equal to that of 25 $\mu g/ml$ of papaverine. The effects were reversible on tissue washing.

The quantitative comparison on guinea pig ileum showed that the mean ($\pm SE$) ratio of antispasmodic ED_{50} concentrations of papaverine and DC was $1:10.5 \pm 0.9$ against acetylcholine (0.1 $\mu g/ml$), $1:8.9 \pm 0.5$ against histamine (0.1 $\mu g/ml$) and $1:10.1 \pm 0.8$ against barium chloride (100 $\mu g/ml$).

2. Isolated uterus : DC, in concentrations of 25 $\mu g/ml$ and above, produced relaxation and/or decrease in spontaneous activity of the oestrinised uterus of the albino rat. The antispasmodic activity of DC on albino rat uterus was approximately 1/10 of that of papaverine against oxytocin (5 $\mu g/ml$), acetylcholine (10 $\mu g/ml$) and 5-HT (5.0 $\mu g/ml$).

3. *Dog tracheal muscle* : DC had no significant effect of its own on tone of the tracheal muscle. Papaverine was nearly twice as potent as DC against spasms induced by acetylcholine (0.1 $\mu\text{g/ml}$) and potassium chloride (10 mg/ml).
4. *Frog's blood vessels* : DC and papaverine had no significant action of their own on the resting perfusion pressure. But both DC (0.25 to 1.25 $\text{mg}/100\text{ g}$) and papaverine (25 to 100 $\mu\text{g}/100\text{ g}$) antagonised barium chloride-induced vasoconstriction, the former being required in nearly 10-12.5 times the doses of papaverine for a near-complete antagonism of the vasoconstriction caused by simultaneously administered barium chloride.

DISCUSSION

Agrawal *et al.* (1) had reported a direct smooth muscle relaxant activity in the total aqueous fraction of alcoholic extract of the seeds. Gambhir *et al.* (3) confirmed their findings and also detected a cholinergic and a cardiotoxic action. The cholinergic activity was subsequently shown chemically and pharmacologically to be due to the presence of the quaternary base, choline (4) and cardiotoxic principle is yet to be isolated and studied.

The alkaline-chloroform fraction of the alcoholic extract of seeds have now been shown to contain a Mayer's positive tertiary base which has been considerably purified as a bromide derivative. However, its chemical characterisation could not be done owing to very low yields. This tertiary base was found to possess a relaxant and antispasmodic action on the smooth muscles of ileum, uterus, blood vessels and trachea of different species of animals, which was similar to the activity of papaverine against various spasmogens. The quantitative comparison showed that the tertiary base bromide was approximately one tenth as potent as papaverine on most of the tissues. The relaxant antispasmodic activity observed earlier by us (3) and Agrawal *et al.* (1) could have, at least partly, been due to the tertiary base now isolated.

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