

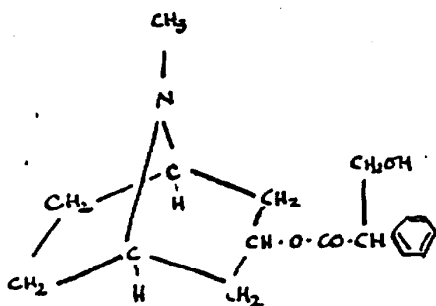
28th December, 1964.

AN EXAMINATION OF THE ALKALOIDS OF BULGARIAN "ATROTAB"
CIGARETTE TOBACCO

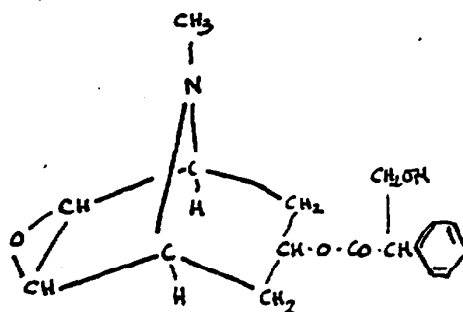
INTRODUCTION

"Atrotab" tobacco is described in a popular article in "Bulgaria Today" (1). It is obtained by grafting tobacco scions (*Nicotiana Tabacum*) onto the wild thorn-apple plant (*Datura Stramonium*) and is said to produce a tobacco variety having the characteristic aroma and flavour of tobacco without containing any nicotine. In place of nicotine, three other alkaloids are reported to be present in the leaves at a concentration of about 0.1%, atropine, hyoscyamine and scopolamine, and these are supposed to endow the tobacco with beneficial medicinal properties.

l-Hyoscyamine is the most commonly occurring of the tropane alkaloids and is isomeric with atropine. Atropine is optically inactive and is actually the racemic form of *l*-hyoscyamine. Since hyoscyamine is racemised exceedingly easily, it is highly probable that atropine is not present in the plant but results from the racemisation of *l*-hyoscyamine during isolation. The structures of hyoscyamine and scopolamine are:



Hyoscyamine & Atropine



Scopolamine

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EXPERIMENTAL

(a) Determination of the nicotine content 10 g. of tobacco were finely ground and refluxed for one hour with 350 ml. 0.05N sulphuric acid, filtered and washed with 500 ml. 0.005N sulphuric acid. The pH was adjusted to 3 - 3.5 and the solution concentrated to about 200 ml. The solution was left to cool in the refrigerator overnight and the precipitate which formed was filtered off. Sodium hydroxide was added to the filtrate to bring it to a pH of 6 - 7 and 2.5 g. magnesium oxide added. The mixture was steam distilled using Frankenburg's method (2) and the distillate examined by U.V. spectroscopy.

The U.V. spectrum showed the absence of any specific absorption at 260 m μ .

(b) Examination of the tropane alkaloids 5 g. of finely ground tobacco were extracted in a Soxhlet with 95% ethanol until no more colour was removed by the solvent. The ethanol extract was evaporated to dryness under reduced pressure and the residue taken up in about 50 ml. 1% hydrochloric acid. The solution was filtered, made alkaline with caustic soda, and extracted three times with 10 ml. portions of chloroform (3). The chloroform extract was washed with water, dried over anhydrous magnesium sulphate and evaporated to dryness. The residue was dissolved in chloroform and made up to 1 ml.

A drop of the chloroform extract was treated with a drop of fuming nitric acid and evaporated to dryness at 100°C. One drop of ethanolic potassium hydroxide was added and an intense purple coloration developed (Vitali Reaction) showing the presence of tropane alkaloids.

Exactly 10 μ l of the chloroform extract were spotted on a thin layer plate of Silica Gel G (250 μ) together with 5 μ l of 1% atropine and 5 μ l of 1% scopolamine. The plate was developed with a mixture of dimethylformamide

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triethylamine, ethanol, ethyl acetate (1:1:6:12) and sprayed (after removal of dimethyl formamide by heating to 110°C under reduced pressure for ¼ hour) with the Dragendorff reagent (Munier modification). The alkaloids appeared as yellow-orange spots on an orange background. The following Rf. values were recorded:

1. Atropine 1% solution, 5 µl :	0.259		
2. Scopolamine 1% solution, 5 µl:	0.626		
3. Chloroform extract, 10 µl :	0.259	0.604	0.705

The chloroform extract showed three spots; the one with the lowest Rf. had a mobility comparable with that of atropine and was of similar size and colour. The two spots of higher Rf. overlapped somewhat and it was not possible to say if there was a spot with a similar mobility to scopolamine. The spot with the highest Rf. showed a purple-blue fluorescence in U.V. light ($\lambda_{\text{max}} = 255 \text{ m}\mu$) before the plate was sprayed with the Dragendorff reagent.

CONCLUSION

"Atrotab" tobacco contains no measurable nicotine (i.e. < 0.02%). It contains hyoscyamine (detected as atropine) in approximately the concentration claimed in an article in "Bulgaria Today". Evidence for the existence of scopolamine is inconclusive.

BIBLIOGRAPHY

1. Bulgaria Today No. 6. June (1963).
2. Frankenburg et al. Anal. Chem. 25 1784 (1953).
3. Paech, K. and Tracey, M. Modern Methods of Plant Analysis Vol. 4. p. 449, Springer-Verlag (1955).

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