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Peroxide-Sulphuric Acid Test as an Indication of the Ripeness and Physiological Activity of Cannabis Resin

SIR,—Duquénois and Negm (1938) have reported a reaction with hydrogen peroxide and sulphuric acid as a very sensitive but not specific enough test for the identification of cannabis. This should not be mistaken for the "Duquénois-Negm" vanillin-acetaldehyde test for cannabis, described in the same paper, which has found a wide application for the identification of hemp resin. No further data on the use of the peroxide-sulphuric acid test for cannabis have been made available since.

To examine the reaction of various types of cannabis resin, 49 samples originating from 11 countries have been analysed by means of the following procedure. Cannabis was extracted by maceration in light petroleum (1 : 20)

TABLE I

Origin	Number of samples analysed	Ripeness	Colour obtained
Germany	5	Unripe	Pink
Switzerland	1	"	"
Yugoslavia	2	Intermediate	"
Morocco	1	"	"
Spain	4	"	Pink to reddish-brown
Cyprus	4	"	"
Greece	6	"	Reddish-brown to brown
Brazil	15	Ripe	Mostly brown
Costa Rica	1	"	Brown
Burma	1	Overripe	Greenish-brown
Cyprus	1	Spoiled	None
Canada	8	"	"

for 24 hr. 0·2 ml. of the extract was left to evaporate in a porcelain dish. To the residue 2 drops of a 20 per cent hydrogen peroxide and 0·5 ml. of concentrated sulphuric acid were added and the dish rotated gently for 1 min. The colour of the liquid was observed after 5 min.

Most samples gave colours which ranged from pink (temperate regions) to brown or greenish brown (mostly tropical samples). But some of the samples did not exhibit any colour at all. The results are summarised in Table I. For comparison, their ripeness has been recorded in the same Table. Full details of constants, based upon an indophenol reaction, are given elsewhere (Grlić, 1961).

To elucidate the differences obtained, the same test was made with pure cannabinolic compounds. Cannabidiol yields a pink product, which in higher concentration appears blood-red. Tetrahydrocannabidiol exhibits a violet colour. Six synthetic tetrahydrocannabinol homologues showed strong brown colours. Synthetic cannabiol showed at first a green colour going quickly

into greenish brown. Cannabidiolic acid acetate reacted to give a colour ranging in various concentrations from orange to pink.

The results have been compared with those obtained by the indophenol method and explained in accordance with the classification of cannabis resin described in previous papers (Grlić, 1961; Grlić and Andrec, 1961). Samples exhibiting a pink colour correspond to the unripe or intermediate type, containing mostly cannabidiol or cannabidiolic acid, which is readily converted into cannabidiol (Schultz and Haffner, 1960). Samples in which the phytochemical conversion process of the cannabinolic constituents was more advanced (ripe cannabis) yield a brown reaction product. This group contains predominantly tetrahydrocannabinols and consequently (Loewe, 1950) exhibits the highest physiological potency. The reaction was greenish brown for overripe samples, containing predominantly cannabinol, the final product of conversion. A negative reaction is exhibited by samples containing mostly disintegration products of cannabinolic constituents.

As it is seen, the peroxide-sulphuric test seems to be suitable as an indication of the progress of the ripening process in hemp resin. In addition, the test seems to be useful for a rapid and rough estimation of the potency of the drug. A highly potent cannabis will exhibit a strong brown colour owing to the presence of tetrahydrocannabinols. Cannabis yielding an orange, pink or red reaction contains precursors of physiologically active constituents. Such hemp may be considered as potentially active, being converted into an active form under favourable conditions. A greenish brown colour or a negative reaction indicates the loss of physiological activity.

Consequently, the proper use of the peroxide-sulphuric test does not appear to lie in its possible identification of the geographical source of drug, but to distinguish cannabis resin of various compositions and potency. This reaction may be of practical use as a simple and rapid substitute for the complicated chemical and biological methods where no quantitative analytical data are required.

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