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Note

Formation of olivetol during gas chromatography of cannabinoids

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Several hundred compounds have been identified as components in various cannabis preparations[1,2]. Two substances not reported, olivetol (3,5-dihydroxy-*n*-pentylbenzene) and olivetolic acid, have been suggested as biogenetic precursors of the cannabinoids possessing the pentyl side-chain[2]. 3,5-Dihydroxyalkylbenzenes are known to occur in species of the *Anacardiaceae*, *Ginkgoaceae*, *Graminaea*, and *Proteaceae*[3], but they have not been reported in the *Cannabaceae*. It therefore appeared worthwhile to investigate samples of cannabis for the presence of olivetol. We were able to isolate and conclusively identify olivetol following gas chromatography (GC) of cannabis extracts as described here. Subsequent work indicated that most if not all of the olivetol resulted from the decomposition of cannabinoids, particularly cannabidiol and cannabigerol, during the chromatographic procedure. Olivetol was previously reported as one of several high-temperature (700°) pyrolysis products of cannabidiol[4].

EXPERIMENTAL

GC was performed on a HydroFlow Series 3000 instrument by direct injection into the packing of a 6 ft. × ¼ in. glass column containing 5% OV-7 on Chromosorb W (Chromatographic Specialties, Brockville, Canada). The injection port temperature was varied between 150 and 300°. Mass spectra were determined at 70 eV on a Hitachi Perkin-Elmer RMU-6L spectrometer using the probe at 180°. Marihuana was Soxhlet-extracted with hexane, the extract was evaporated to dryness and the residue was dissolved in methanol for GC examination. Fractions were collected from the gas chromatograph by placing the tip of a disposable pipette over the extinguished flame-ionisation detector jet, at the appropriate retention time. Fractions and derivatives were examined by mass spectrometry (MS). The olivetol fraction was methylated by rinsing the contents of a pipette into a small test tube with about 50 μl of 0.4 *M* trimethylanilinium hydroxide in methanol (prepared and donated by Dr. K. K. Midha of these laboratories) and injecting a portion of the resulting solution into the gas chromatograph. The olivetol fraction was bistrifluoracetylated by rinsing the contents of the pipette into a small test tube with about 100 μl of trifluoracetic anhydride and injecting the solution into the gas chromatograph. Olivetol was mixed in turn with α-pinene, limonene, linalool, caryophyllene and *p*-menthadienol. The mixtures were

injected into the gas chromatograph at a port temperature of 300°. Chromatograms were determined at various oven temperatures between 150 and 250°.

RESULTS AND DISCUSSION

Small signals were observed in chromatograms of marihuana extracts at retention time (t_R) 4.6 and 6.0 min at oven temperatures 185 and 175°, respectively. These t_R values corresponded with those found under the same conditions for authentic olivetol. Injections of a mixture of extract with olivetol gave single sharp peaks at these t_R values. The mass spectrum obtained from this marihuana fraction was essentially the same as that of authentic olivetol: *m/e* (%) 180 (M^+, 30), 138 (17), 137 (18), 125 (13), 124 (100), 123 (50), 95 (10), 77 (13), 69 (14), 67 (12), 55 (12). The formation of the base peak at 124 and other ions from 3, 5-dihydroxybenzenes has been discussed previously[5]. Further proof of the identification of olivetol was made by derivatization of the GC fractions. Methylation was effected with trimethylanilinium hydroxide in methanol; this reagent efficiently flash-methylates the phenolic group of cannabinoids[6]. The olivetol was completely converted into a compound with the same t_R (7.8 min at 150°) as olivetol dimethyl ether. The mass spectrum obtained was indistinguishable from that of authentic material[7]: *m/e* (%) 208 (M^+, 21), 166 (14), 165 (20), 153 (12), 152 (100), 151 (15), 123 (10), 121 (12), 91 (11), 86(11), 84 (17), 57(11), 55 (12). The reaction of the olivetol fraction with trifluoracetic anhydride gave a product of t_R 6.6 min at 190° identical with that from authentic olivetol treated similarly. The olivetol peak no longer appeared. The product appears to be the bistrifluoracetate: a mass spectrum of a collected fraction showed a weak signal at *m/e* 372 (the molecular ion) but was otherwise essentially the same as that of the olivetol to which the diester is rapidly hydrolysed.

The possibility that the olivetol (estimated to be about 0.03% by weight of the dry marihuana) arose by decomposition of cannabinoids was investigated by progressively lowering the injection port temperature. The peak height of the signal due to olivetol progressively decreased in relation to the main cannabinoid signals and it could not be detected below 150°. Cannabidiol and cannabigerol were found to give a GC peak corresponding with that of olivetol at injection port temperatures above about 200°. The peak increased in relative intensity up to 300°, the maximum temperature investigated. No such peak was observed with Δ^9-tetrahydrocannabinol or cannabinol at temperatures up to 300°.

We considered the possibility that complex molecules of cannabinoid-like structure could arise during GC by the reaction of olivetol with various terpenes present in cannabis. This was investigated by mixing the olivetol in turn with α-pinene, limonene, linalool, caryophyllene and *p*-menthadienol and injecting the mixture into the gas chromatograph at an injection port temperature of 300°. Only the starting materials could be detected. The absence of materials of greater retention time indicates that this process is absent or negligible.

CONCLUSION

Cannabidiol and cannabigerol, both dihydric phenols, decompose with the formation of olivetol during GC at injection port temperatures over 150°. The

possibility of this and other pyrolytic processes occurring during routine GC analyses should be considered especially when novel, trace components of cannabis are being investigated.

REFERENCES

1 R. N. Smith, L. V. Jones, J. S. Brennan and C. G. Vaughan, *J. Pharm. Pharmacol.*, 29 (1977) 126.
2 R. Mechoulam, N. K. McCallum and S. Burstein, *Chem. Rev.*, 76 (1976) 75.
3 D. D. Ridley, E. Ritchie and W. C. Taylor, *Aust. J. Chem.*, 23 (1970) 147.
4 F. J. E. M. Küppers, C. A. L. Bercht, C. A. Salemink, R. J. J. C. Lousberg, J. K. Terlouw and W. Heerma, *J. Chromatogr.*, 108 (1975) 375.
5 J. L. Occolowitz, *Anal. Chem.*, 36 (1964) 2177.
6 K. Bailey and D. Gagné, *J. Pharm. Sci.*, 64 (1975) 1719.
7 K. Bailey, *Can. J. Chem.*, 52 (1974) 2136.