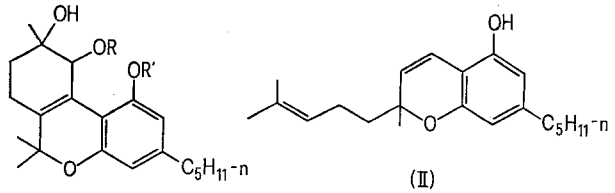
m.p. 170–172° and gave a positive test with Gibbs reagent. We have isolated a compound with similar properties to those of cannabitriol from a sample of Jamaican ganja and propose the structure (I) for this compound.

The benzene extract from the dried leaves, twigs and flowering tops was partitioned between light petroleum and 10% aqueous methanol. The neutral portion of the aqueous methanolic extract was chromatographed on alumina, and the material eluted with 20% ethyl acetate in benzene was subjected to further fractionation by PLC on silica. The major UV-active band gave a compound (0.025% from dried weight of plant material) identical by m.p., published IR-spectra (Nujol and KBr) and colour reactions with cannabitriol.

Cannabitriol, $C_{21}H_{30}O_4$ (analysis and mass spectrum), m.p. 171–173°, $[\alpha]_D$ −107° ($CHCl_3$), had absorption maxima in the UV (EtOH) at 231 and 279 nm (ε 25,000 13,800 respectively) indicative of the presence of a styrene type chromophore [cf. cannabichromene (II): λ_{max} 228 and 280 nm (ε 25,100 and 8,900 respectively)[4]]. Addition of base to the ethanolic solution led to the formation of a new maximum at 326 nm (ε 7,200).



(I) R = R' = H
(III) R = H; R' = Me
(IV) R = R' = Ac.

(II)

Treatment of cannabitriol in methanol with a trace of sulphuric acid at reflux for 2 h led to the isolation of cannabinol (69%) which could not be crystallized but was identified by its TLC behaviour and by comparison of its IR- and NMR-spectra with the published data[5]. This conversion establishes the carbon skeleton of cannabitriol. The location in ring A of the double bond and the two remaining oxygen atoms became evident from an examination of the NMR-spectra of the methyl ether (III) and the diacetate (IV).

The methyl ether (III), $C_{22}H_{32}O_4$, m.p. 111–113°, prepared by overnight treatment of cannabitriol with diazomethane in ether/methanol, had λ_{max} 227 and 277 nm (ε 27,600 and 14,800 respectively). The NMR-spectrum ($CDCl_3$) confirmed the presence of a primary methyl (triplet at δ 0.90), 3 tertiary methyls [singlets at 1.30 (3H) and 1.38 (6H)], a methoxy group (3.92) and 2 aromatic protons (6.40 and 6.43). Deuterium exchange revealed a singlet at 4.27 attributable to C*H*OH. The appearance of this signal indicates that there are no protons on the adjacent carbons.

The gummy diacetate (IV), obtained by treatment of cannabitriol with acetic anhydride and pyridine overnight at room temperature, partially regenerated cannabitriol on attempted purification by PLC. It had hydroxy absorption in the IR. The NMR-spectrum ($CDCl_3$) showed the presence of 5 methyl groups: 1 as a triplet at 0.89 (side-chain methyl), 3 as singlets at 1.13, 1.20 and 1.40, and 2 as acetates at 2.03 and 2.25. A proton at the base of an acetate appeared as a broadened singlet at 5.68 and 2 nonequivalent aromatic protons as doublets (J 2Hz) at 6.47 and 6.69. The above evidence can only be interpreted in terms of the structure (I) for cannabitriol. Cannabitriol is recovered unchanged from attempted oxidation with manganese dioxide or on treatment with acetone and anhydrous copper sulphate.

An ester of cannabitriol, linked through the C-1 hydroxy group with cannabidiolic acid, has been reported as a constituent of ganja of Turkish origin[6].

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Detection and Substitution-Pattern Determination of Guanidines[1]

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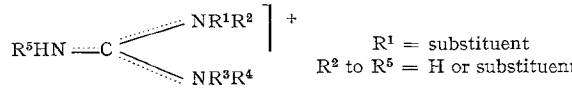
Summary. The guanidine function can be detected and its substitution pattern determined taking into account the ^{1}H-NMR signals of the N–H and N–C–H groups. Satisfactory results were obtained with mono- to penta-substituted guanidines (as picrate salts).

Guanidine and its derivatives bearing up to 5 substituents occur in plants or animals[2]. The guanidine function was usually detected by pKa measurements[3] and colour tests[4], and with more limitations by electrophoretic mobility[5], IR-[6] or mass[7] spectra. Colour tests[4] and IR-absorption[8], and less frequently electronic[9] or fluorescence[10] spectra were used for direct determination of the number and location of substituents; the scope of these methods is limited, e.g. IR-spectra do not distinguish N_1-mono from N_1,N_1-di-substituted guanidines. Besides, colour tests[11,12] may lead to contradictory or erroneous conclusions. Even the non-direct methods based on degradations[2,4] may give ambiguous results[13].

We report here a direct method for both purposes; it is based on the ^{1}H-NMR signals of N–H and N–C–H of the guanidine group (as picrate salt). The method gave good results with numerous synthetic or natural compounds of the types shown in the scheme.



The spectrum and integration curves (60 MHz) of the guanidinium picrate (0.15 mmol) were recorded at 35 °C in acetone (0.5 ml) using tetramethylsilane as internal standard; after addition of methanol (75 µl) the measurements were repeated. The same NMR measurements were then performed dissolving the guanidinium picrate

(0.15 mmol) in acetone-d_6 (0.5 ml); methanol-d_4 (0.2 ml) was added and after 24 h (N–H to N–D) the curves were recorded again. The singlet (δ 8.6–8.8) of the picrate anion was taken as proton-counting reference; the number of N–H resulted directly from the integration curves or from the difference with those obtained after addition of methanol-d_4. The number found for N-H was usually within 0 to -10% of the correct one.

The spectra do not show separated signals for E–Z isomers[14]. The N–H signals are generally characterized by: δ-values in the range 6.7–8.2; half-band width ($W_{1/2}$) 5–18 Hz; they disappear with methanol-d_4; δ-values are not changed or are slightly decreased (less than 0.1 ppm) by methanol. An aryl substituent produces: a strong downfield shift of the Ar–N–*H* signal; a small effect on the protons of the other N-atoms; the N–H resonances disappear ('averaged' with MeO–*H*) or are strongly broadened by methanol.

The aromatic-H signals are distinguished because they are not removed by methanol-d_4. The N–H signals of amines (as picrate salts) are usually very broad ($W_{1/2}$ > 40 Hz) and disappear by methanol. The O–H ($W_{1/2}$ ca. 2 Hz) or N–H ($W_{1/2}$ > 40 Hz) signals of phenols and carboxamides are shifted downfield (0.2–0.3 ppm) by methanol.

After the presence of the guanidinium group has been ascertained, its substitution pattern results in many examples from the N–H signals in the spectra measured in plain acetone. Thus, an example ($R^1 = R^3$ = cyclohexyl; $R^2 = R^4 = R^5 = H$) exhibits 4 N–H distributed in 2 signals (δ 7.06 and 7.26; area ratio 1:3) partially superposed; these data indicate non-equivalent N–H groups, and therefore the compound is a N_1,N_2-disubstituted guanidinium. The observed area ratio is due to superposition of the lower-field component of the splitted N–H signal (doublet; two H–N–C–H) with the NH_2 singlet. The splitting is confirmed by decoupling with ferric chloride.

The scope is widened, including the results in acetone-d_6, which allows the observation of the N–C–H signals at δ 3.0–4.3; an adjacent aryl group (N–C*H*–Ar) produces a downfield shift (δ 4.5–4.9). The method now takes into account: number of guanidinium N–H; occurrence of a singlet or more N–H signals arising from non-equivalence or spin-spin coupling with N–C–H; number of N–C–H multiplets simplified by methanol-d_4.

One example is given in detail ($R^1 = R^2$ = ethyl; R^3 = methyl; R^4 = H; R^5 = benzyl) which gives a sharp signal at δ 7.33 that in its base is partially superposed with a broad signal (total area 7 H). After addition of methanol-d_4 it remains a 5H-singlet (aromatic-H), indicating that the compound is a tetra-substituted guanidinium. Besides, methanol-d_4 simplifies two doublets to give the corresponding singlets at δ 3.00 and 4.55; this implies that two of the substituents are located on N-atoms bearing a proton responsible of the N–C–H splittings. Then, the compound is a N_1,N_1,N_2,N_3-tetra-substituted guanidinium.

Some of the compounds were also measured using plain and hexadeuterated dimethylsulphoxide as solvents; the results were generally similar to those quoted above. These solvents are mainly useful for low soluble picrates (e.g. $R^1 = R^2$ = methyl; $R^3 = R^4 = R^5 = H$).

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Double Stranded Ribonuclease Activity in Human Lymphocyte Nuclei

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Summary. Ribonuclease activity directed against the synthetic duplex polyrC:polyrI was detected in nuclear extracts from both unstimulated and PHA-stimulated human lymphocytes. In the latter cells, the activity was about twice that of small lymphocytes.

It has been shown that human small lymphocytes synthesize mainly unmethylated rapidly sedimenting RNA molecules which hybridize very efficiently to DNA in vitro and are bound to polyadenylic sequences in a proportion up to 20%[1,2]. It was thus concluded that the major portion of the RNA synthesized in these cells is the heterogeneous nuclear RNA. Since it has been shown by several authors that a significant portion of nuclear RNA of the heterogeneous type in animal cells is in an RNase-resistant form with properties of double-stranded

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