GLC failed to reveal any additional peak compared to reference samples of DDT and sensitizers. The suspensibility was determined by WHO/SIF/1.R_3 (1967) method[8]. Glass cylinders wrapped in black carbon paper were used for the purpose. To avoid sensitizer interference during the colour change at end point of titration, the solution, after precipitating AgCl, was extracted with petroleum ether. This removed the sensitizers. pH of 1:5 (compound: water) suspensions was measured using a line operated, glass electrode pH meter. Sieving requirement after accelerated storage was determined as per WHO/M/4 method[8]. Colour after storage (since sensitizers attain colour on exposure to light) and phytotoxicity symptoms of compounds were visually observed. Phytotoxicity observations were made against rice, cowpea, wheat, moong and a few ornamentals.

Results and discussion. The results in the Figure reveal that both diphenylamine and diethylaniline enhance the photodegradation of DDT. The enhancement is initially more; almost 30–40% more DDT being lost in sensitized compounds compared to DDT in the control. The subsequent slowing down of the rate could be attributed to the volatile nature of these amine sensitizers. The relative efficiency of these sensitizers seems to be influenced by plant surfaces. An overall better performance is also apparent with diphenylamine.

It is seen from observations reported in the Table that incorporation of diphenylamine or diethylaniline in the compounds neither affects their storage stability, pH, sieve requirement and colour, nor introduces any phytotoxicity. However, the addition of diphenylamine lowers the suspensibility, which, though still marginally above the WHO prescribed limit of 60%, could be a slight cause of concern.

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A Possible Chemical Basis for the Higher Mutagenicity of Marijuana Smoke as Compared to Tobacco Smoke

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Summary. The results of comparative analyses of polynuclear aromatic hydrocarbons in marijuana and tobacco smoke indicate a considerably higher content of potential carcinogens in the former. A model experiment involving Δ^9-tetrahydrocannabinol suggests that the pyrolysis products of cannabinoids are major contributors to the polynuclear aromatic hydrocarbons.

Certain adverse effects of marijuana smoking observed both clinically and in laboratory experiments are bronchial irritation and the action of smoke in lung tissue that produces biochemical and cellular abnormalities characteristic of the early stages of cancer[3]. Leuchtenberger et al.[4–6], studied the effects of marijuana and tobacco smoke, passed under standard conditions over mouse[4] and human[4,6] lung explants, on DNA synthesis and chromosomal complement. More pronounced abnormalities from marijuana smoke were observed.

Obviously, a chemical basis must exist for the above observed phenomena. Although many details concerning the complex chemistry of marijuana smoke have been so far unavailable because of purely methodological reasons (e.g., the problems of chromatographic resolution and identification methods), we have chosen to investigate the occurrence of the potent carcinogens, polynuclear aromatic hydrocarbons, in marijuana smoke condensate as the primary objective. Detailed structural studies performed in our laboratory (using liquid-chromatographic methods, NMR-spectroscopy, and the combination of high-resolution gas chromatography-mass spectrometry) resulted in identification of over 150 polynuclear aromatic hydrocarbons in marijuana smoke condensate. The results were further compared with those obtained with smoke condensate from an equal amount of standard high-tar tobacco cigarettes.

Cigarettes prepared from equal weights of Mexican marijuana (standard material obtained from the National Institute of Mental Health, Rockville, Maryland; the content of Δ^9-tetrahydrocannabinol: 2.8%) and standard tobacco cigarettes (from Tobacco-Health Research Institute, University of Kentucky, Lexington, Kentucky) were smoked by means of a standard smoking machine[7] and smoke collected. Approximately 2,000 cigarettes from each were used in the pilot study. Smoke condensates were further processed by a modified previously reported fractionation procedure[8].

Total weights of the polynuclear aromatic fractions containing 3 rings and greater were 73.7 mg and 56.9 mg for marijuana and tobacco, respectively. High-resolution gas chromatography using a glass capillary column and precolumn concentration technique (Figure 1) shows the profiles of polynuclear aromatic hydrocarbons for both materials within the volatility range of tricyclic to hexacyclic molecules. The profile comparison indicates a larger proportion of heavier polynuclears encountered in mari-

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Selected polynuclear aromatic hydrocarbons found in smoke condensates[a]

Compound	Molecular weight	Amount µg/100 cigarettes Marijuana	Tobacco
Pyrene	202	6.6	6.8
Benzo [*a*] fluorene	216	4.2	4.9
Benzo [*a*] anthracene	228	3.3	2.6
Chrysene	228	5.5	5.1
Benzo [*j*] fluoranthene	252	3.0	2.1
Benzo [*k*] fluoranthene	252	1.1	1.2
Benzo [*a*] pyrene	252	2.9	1.7
Perylene	252	0.9	not detected[b]
Dibenz [*a, i*] anthracene	278	0.3	not detected
Benzo [*ghi*] perylene	276	0.7	0.3
Anthanthrene	276	0.5	not detected
7 Isomeric compounds with m.w. 276[c]		0.3	not detected
		0.3	not detected
		0.6	not detected
		1.0	0.3
		0.3	not detected
		0.4	not detected
		0.4	0.2
		0.4	not detected
2 Dibenzopyrenes	302	0.5	not detected
		0.3	not detected

[a] Alkylated compounds of almost all major heavier parent polynuclears have also been resolved and detected, but their identities cannot be presently determined due to unavailability of reference compounds. It should be noted that many methyl derivatives of the compounds with m.w. 276 were found in marijuana condensate, but not in tobacco condensate. [b] Presence of these compounds in tobacco smoke condensate is not ruled out in the presented comparisons and they might be detectable at considerably lower concentration after enrichment. [c] These may be any of the following: indeno [1,2,3-*cd*] pyrene, indeno [1,2,3-*cd*] fluoranthene, aceperylene, phenanthro [10,1,2,3-*cdef*] fluorene, acenaphth [1,2-*a*] acenaphthylene, dibenzo [*b, mno*] fluoranthene, dibenzo [*e, mno*] fluoranthene, dibenzo [*f, mno*] fluoranthene, or benzoderivatives of aceperylene and acefluoranthylene.

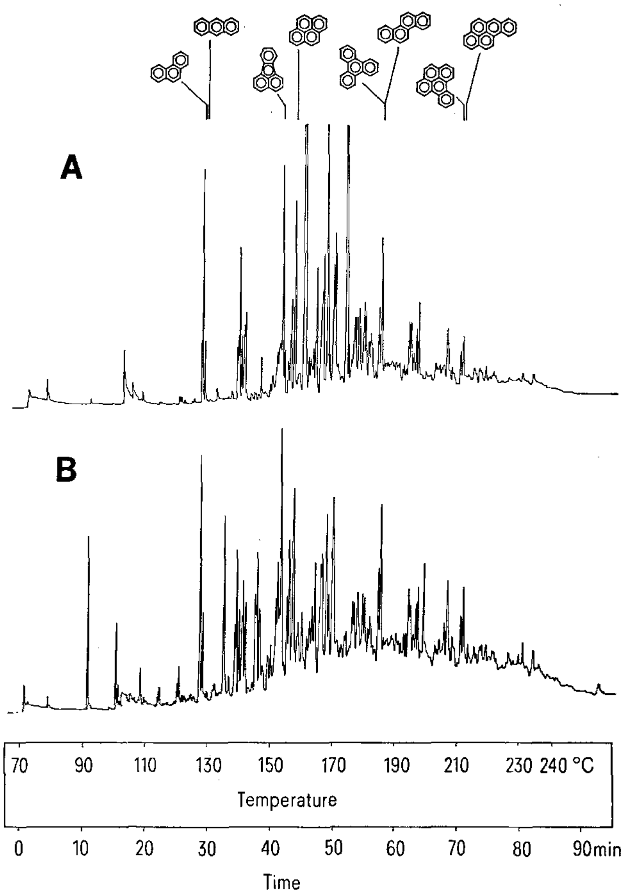

Fig. 1. Comparative high-resolution gas chromatograms of the polynuclear aromatic hydrocarbon fraction from tobacco (A) and marijuana (B) smoke condensate. Experimental conditions: 11 m× 0.26 mm, i.d. glass capillary column coated with SE-52 silicone gum; solvent-free direct injection as described in [16].

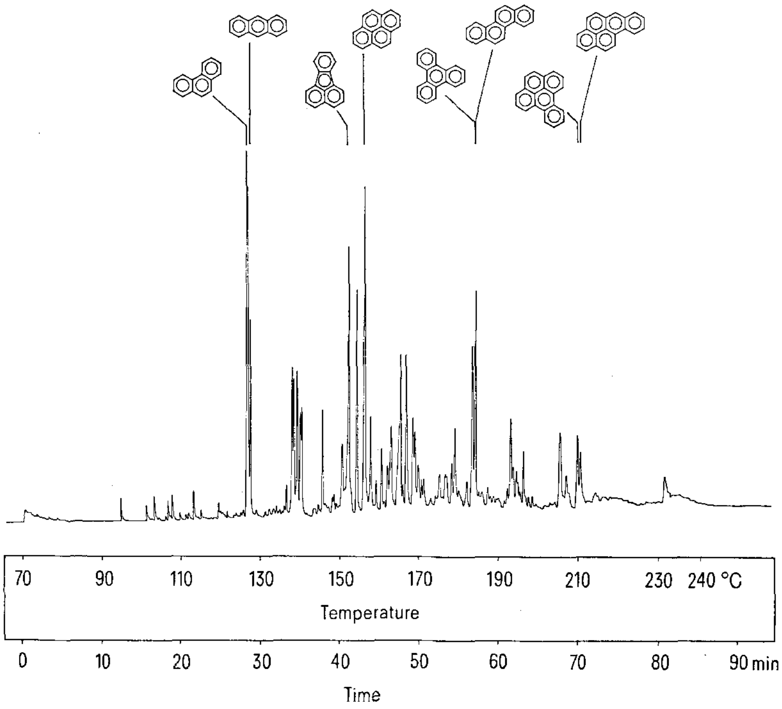

Fig. 2. Chromatogram of the polynuclear aromatic hydrocarbon fraction obtained from the pyrolysate of Δ^9-tetrahydrocannabinol. Experimental conditions as in Figure 1.

juana smoke compared to tobacco, in addition to some qualitative and quantitative changes within the profiles.

Many heavier mixture constitutents that are not directly seen in Figure 1 were selectively enriched by liquid chromatography[8] and spectrally identified. While the detailed analytical data on all mixture constituents are reported elsewhere[9], the Table lists some selected potentially important polycyclics. It should be noted that the well-known carcinogen benzo [a] pyrene is enhanced in marijuana smoke by 70% over that present in tobacco smoke. In addition, indeno [1, 2, 3-cd] pyrene has been known as a carcinogen[10] and, also, dibenzopyrene has been reported[11] as effective in producing respiratory cancer in hamsters. Carcinogenicity of many other polycyclics found in this work[9] is not presently known, because most of them have never been synthesized or isolated in a pure state for biological experiments.

Further work was directed toward a possible explanation for higher amounts of polynuclears in marijuana smoke, their origin and pyrosynthesis. It has been previously suggested that phytosterols[12] and terpenes[13] may function as specific precursors for polynuclear aromatic hydrocarbons. However, determination of the total sterol fraction by a modified procedure of KELLER et al.[14] yielded lower figures for marijuana as compared to dry tobacco leaf.

Due to their cyclic structures and high content in cannabis plants (in some cases up to 5–10% of total weight[15]), cannabinoids could be a major source of polynuclear aromatic hydrocarbons. To test this hypothesis, we have carried out a model pyrolysis experiment, in which 0.27 g of Δ^9-tetrahydrocannabinol was pyrolyzed in a quartz tube at 850 °C in a stream of helium, the pyrolytic products were collected (yield 0.20 g) and carried through the fractionation procedure used previously for condensates. There is a resemblance of the chromatogram obtained from the polynuclear fraction of the pyrolyzate (Figure 2) with the total smoke profile (Figure 1, B). Naturally, many other constituents of marijuana may contribute of the formation of polynuclear aromatic hydrocarbons. In addition to other cannabinoids, to a lesser degree, nonpolar higher terpenes that are present in marijuana extracts in greater abundance than in tobacco (M. L. LEE and M. NOVOTNY, unpublished experiments), are the most likely candidates.

Although higher mutagenicity of marijuana smoke[4–6] as compared to tobacco smoke may not necessarily mean higher carcinogenicity (evidence from animal studies is needed), the correlation between these biological phenomena and the information on major chemical carcinogens presented in this work is highly suggestive. Furthermore, the presence of co-carcinogens, other tumor promoters, irritants, etc. in marijuana smoke must be considered in future studies.

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Some Aspects of the NMR-Spectra of Aporphine and Phenanthrene Alkaloids[1]

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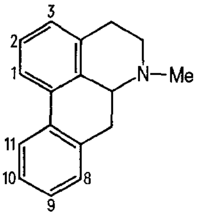
Summary. The C-11 proton of an aporphine possessing a C-1,2 methylenedioxy group falls relatively upfield between δ 7.47 and 7.86. Additionally, the i.c.s. for the two protons of the methylenedioxy group is large (4–12 Hz). The presence of a methylenedioxy at C-3,4 in a phenanthrene alkaloid also results in an upfield shift (δ 8.95–9.00) of the C-5 proton.

Although useful NMR correlations have been drawn to assist in locating aromatic hydrogens as well as methoxyl substituents on the aporphine skeleton[2], no generalizations have so far been made in the assignment of methylenedioxy groups. However, careful examination of published data has now allowed some firm correlations concerning this dioxygenated substituent.

The five possible locations for a methylenedioxy in an aporphine are 1,2, 2,3, 8,9, 9,10 and 10,11. The presence of a C-1,2 methylenedioxy group in an aporphine (**1**) or a noraporphine free base is evidenced by an upfield shift of the C-11 proton which appears in the range δ 7.47 to 7.86 (Table). But when a hydroxyl or a methoxyl group is at C-1, the C-11 proton signal is further downfield, between δ 7.80 and 8.21.

Additionally, the internal chemical shift for the two protons of the methylenedioxy group is large (4–12 Hz) when the group is located at C-1,2 (Table), and small (2–4 Hz) when at C-2,3. Two aporphines with a methylenedioxy group at C-2,3 are known, namely ocokryptine (1,10-dimethoxy-2,3-methylenedioxy-11-hydroxyaporphine) and *O*-methylocokryptine (1,10,11-trimethoxy-2,3-methylenedioxyaporphine), with internal chemical shift values of 4 and 2 Hz, respectively[3].



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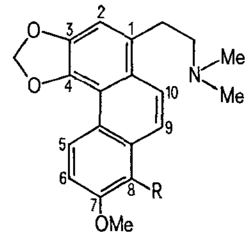

2, R = H
3, R = OMe