Table IV. Percent Aqueous [^{14}C]Tirpate Metabolites and Effect of Acid, Glucosidase, and Sulfatase Treatment

		% hydrolyzed[b]		
Time	Aqueous[a]	Acid	Glucosidase	Sulfatase
0 hr	7.5	50	11	8
3 hr	13	51	7	11
9.5 hr	19	55	8	10
16.5 hr	31	53	8	5
34 hr	46	53	5	7
3 days	64	47	1	
6 days	80	45		
12 days	85	40		3

[a] Percent of total. [b] Percent of aqueous fraction hydrolyzed as detailed in the Experimental Section.

compounds released by sulfatase and glucosidase indicated that they were very polar. The sulfatase hydrolysate contained low levels of a compound with a similar R_f to the Tirpate monosulfoxide at 9, 16, and 34 hr following the treatment. The yields from enzyme hydrolysis were too low to permit further qualitative analysis.

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Cannabis Smoke Condensate. Identification of Some Acids, Bases, and Phenols

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The acids, bases, and phenols were chemically separated from the smoke condensate of 2638 marijuana cigarettes and semiquantitatively analyzed by GC and GC–MS. The following compounds were identified: acids—hexanoic, heptanoic, octanoic, benzoic, salicylic, hexadecanoic, heptadecanoic, and octadecanoic; bases—dimethylamine, piperidine, pyridine, 2-methylpyridine, pyrrole, 3-(and/or 4-) methylpyridine, and dimethylpyridine; phenols—phenol, cresols, quaicol, catechol, hydroquinone, *p*-hydroxyacetophenone, scopoletin, and/or esculetin.

Despite the fact that smoking has been the preferred method of ingestion of *Cannabis* for decades, it is only in the last 6 or 7 years that attention has been directed to the determination of those compounds generated and/or transferred during the smoking process. Heretofore, the major thrust has been directed to the fate of the cannabinoids under the pyrolytic conditions of smoking (Mechoulan, 1973, an excellent review; Fish and Wilson, 1969) and only recently have reports appeared concerning the identification of other compounds present in the smoke condensate. Identified by gas chromatography–mass spectrometry (GC–MS) were long-chain hydrocarbons (Adams and Jones, 1973) while phytosterols were shown to be present in the smoke condensate of *Cannabis* cigarettes by gas chromatography (GC) (Adams and Jones, 1975). Other compounds identified include several acids and phenols (Fentiman et al., 1973) as well as carbazole, indole, and skatole (Zamir-ul Haq et al., 1974).

The latter two studies employed GC–MS for separation and identification and preliminary separations by chemical methods were employed in all studies reported. As part of our continuing study of *Cannabis* and its smoke condensate, we now wish to report the classical chemical separation, GC, and, in some cases, GC–MS identification of some acids, bases, and phenols isolated from *Cannabis* smoke condensate.

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EXPERIMENTAL SECTION

The procedure for the preparation of cigarettes and method of smoking and collection of condensate has been previously described (Adams and Jones, 1973). The bases, phenols, and acids from the smoke condensate of 2638 cigarettes were extracted and analyzed as follows.

Bases. The methylene chloride–acetone solution (1:1, 3 l.) containing the smoke condensate was shaken with 10% HCl (1 l.). The aqueous acid solution was washed with ether (2 × 500 ml) and then continuously extracted with ether for several days. The ether washes and extracts were combined with the methylene chloride–acetone solution and reserved for the extraction of phenols and acids. The aqueous acid solution was cooled to 5–10°C, covered with a layer of ether (1 l.), and made alkaline with solid sodium hydroxide. The alkaline solution was extracted with several portions of ether and then continuously extracted for 5 days to remove final traces of amines. The ether extracts were re-extracted with 5% HCl (1.5 l.) and the acid solution was continuously extracted with ether until the extracts were colorless. The acid solution was then stripped on a

Table I. Percentages of Basic, Phenolic, and Acidic Fractions in *Cannabis* Smoke Condensate

Fraction	Weight, g	%
Bases	1.0	4.8
Phenols	0.96	4.6
Acids	1.57	7.5
Neutrals	17.4	83.1
Total	20.93	100.0

roto-evaporator at 45°C (reduced pressure) and the residue was further dried at room temperature (0.005 mmHg) for 3–4 hr to give 1.47 g of amine hydrochlorides.

A 0.53-g sample of the hydrochlorides was dissolved in distilled water (5 ml). GC analysis was accomplished by using a 10 ft × ⅛ in. stainless steel column packed with 28% Pennwalt 223 + 4% KOH on Chromosorb R, 80–100 mesh. Analytical samples were injected on-column without the use of a fore-column (see below); the small samples were neutralized and dried by the column packing. A Beckman GC-45 gas chromatograph equipped with a FID was used with the following operating conditions: column temperature, 125°C; detector temperature, 250°C; inlet, 150°C; flow rate (He), 27 ml/min.

GC/MS was performed using a Beckman GC-5 gas chromatograph coupled via a Biemann molecular separator to an AEI MS-12 mass spectrometer. Samples (10 μl) of the aqueous solution of hydrochlorides were injected into a 7 ft × 6 in. glass forecolumn of which the first 2 in. were packed with powdered soda lime for liberating the amines and the remaining 5 in. were packed with ascarite for the absorption of water (Kaburi et al., 1969). A 7 ft × ⅛ in. stainless steel column packed with 28% Pennwalt 223 + 4% KOH on Chromosorb R, 80–100 mesh, followed the forecolumn for separation of the free bases. The column was operated isothermally at 100°C; inlet, 115°C; detector, 200°C. Helium was used as carrier gas and the flow was split 10:1 after the column with one part going to a thermal conductivity detector and ten parts to the mass spectrometer.

Phenols. The extracts containing the phenols, acids, and neutrals were evaporated and the residue was washed with 5% aqueous NaOH (2 × 100 ml). The aqueous solution was exhaustively extracted with ether to remove the neutrals and was then treated with HCl followed by $NaHCO_3$ to give a slightly alkaline solution. The phenols were extracted with ether and the extracts were dried ($CaCl_2$), filtered, and evaporated. The residue was dissolved in anhydrous ethanol and reevaporated at 40°C and reduced pressure to remove traces of water. A total of 0.96 g of a dark oil was recovered.

A 10-mg sample of the oil was silylated using *N,O*-bis-(trimethylsilyl)trifluoroacetamide and the derivatized sample analyzed by GC using a 10 ft × ⅛ in. stainless steel column packed with 5% OV-17 on Diatoport S, 60–80 mesh. Samples were injected on-column and a thermal conductivity detector was used. The column temperature was programmed from 60 to 300°C at 7.5°C/min; detector temperature, 325°C; inlet temperature, 315°C; flow rate (He), 28 ml/min.

Acids. After the extraction of phenols, the alkaline solution containing the smoke condensate acids was made acidic with HCl and then exhaustively extracted with ether. The ether extracts were reextracted with 5% aqueous NaOH (4 × 100 ml) and the combined alkaline extracts were saturated with NaCl and exhaustively extracted with ether to remove nonacidic impurities. The aqueous solution was made acidic with HCl and extracted with ether, and the extracts were dried ($CaCl_2$), filtered, and evaporated to yield 1.57 g of acids.

Table II. Amines Identified in *Cannabis* Smoke Condensate[a]

Peak no.	Base	Ret. time, min	%	M^+ *m/e* (rel. abund.)[b]
1	Dimethylamine	1.6	4	
14	Piperidine	14.7	2	
15	Pyridine	16.5	43	79 (100)
16	2-Methylpyridine	24.5	16	93 (100)
17	Pyrrole	26.8	2	
18	3-(and/or 4-) Methylpyridine + dimethylpyridine	35.3	18	107 (32), 93 (100)

[a] Column: 10 ft × ⅛ in. stainless steel, 28% Pennwalt 223 + 4% KOH on Chromosorb R, 80–100 mesh; temperature, 125°C; detector (FID) temperature, 250°C; inlet temperature, 150°C; flow (He), 27 ml/min. [b] For GC–MS conditions, see Experimental Section.

A second work-up of smoke condensate acids was used for GC analysis. The smoke condensate of 100 cigarettes dissolved in methylene chloride (100 ml) was extracted with 5% aqueous $NaHCO_3$ (100 ml). The aqueous extract was washed with methylene chloride (4 × 50 ml) and ether (2 × 100 ml), and was then made acidic with concentrated HCl. The acidic solution was extracted with ether (4 × 50 ml) and the extracts were dried ($MgSO_4$), filtered, and evaporated. The residue was esterified by refluxing with 5 ml of BF_3–MeOH (14%, v/v) for 5 min. The mixture was cooled, poured into 25 ml of ether, and washed with 5% aqueous $NaHCO_3$ (3 × 25 ml). The combined aqueous washes were back-extracted with ether (3 × 25 ml) and the combined ether layers were dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in 1 ml of ether for GC analysis.

Samples of the methyl ester solution were injected on-column into a 10 ft × ⅛ in. stainless steel column packed with 2% OV-17 on Gas-Chrom Q, 80–100 mesh. The column temperature was programmed from 60 to 240°C at 5.6°C/min; detector temperature, 325°C; inlet temperature, 275°C; flow rate (He), 25 ml/min; FID.

Neutrals. The ether extracts of the neutrals (see work-up of phenols) were evaporated at reduced pressure. The residue was dried by adding anhydrous ethanol and again evaporating under reduced pressure to yield 17.4 g of neutrals.

RESULTS AND DISCUSSION

The percentages of the basic, phenolic, acidic, and neutral fractions in the smoke condensate are listed in Table I. For the purpose of calculating the percentage of bases, the weight of the amines was estimated to equal 70% of the weight of the amine hydrochlorides or 1.0 g. The estimate was based on the average molecular weight of the amines listed in Table II individually weighted for their percentages. The total percentage of the acids and phenols, 12%, is in close agreement with the 11% value obtained previously (Fentiman et al., 1973). Individual components of the basic, phenolic, and acidic fractions were identified by GC by comparison of retention times and enrichment of the unknown sample with authentic reference compounds. In addition, when in doubt, further substantiation for the identities of some of the bases was sought by the use of GC–MS. All of the compounds identified have also been found in tobacco smoke (Stedman, 1968, and references therein). Percentages of the individual components are based on relative peak areas in the chromatograms and are semiquantitative. It was assumed that each peak used in

Table III. Silylated Phenols Identified in *Cannabis* Smoke Condensate[a]

Peak no.	Phenol	Ret. time, min	%
5	Phenol	5.3	0.6
8	Cresols	6.8	1.2
14	Guaicol	9.5	0.5
17	Catechol	10.1	3.1
24	Hydroquinone	11.7	0.6
34	*p*-Hydroxyacetophenone	14.8	3.7
58	Scopoletin and/or esculetin	25.6	15.1

[a] Column: 10 ft × ⅛ in. stainless steel, 5% OV-17 on Diatoport S, 60–80 mesh; temperature programmed, 60–300°C, 7.5°C/min; detector (TC) temperature, 325°C; inlet temperature, 315°C; flow (He), 28 ml/min.

Table IV. Acids Identified in *Cannabis* Smoke Condensate as Methyl Esters[a]

Peak no.	Acid	Ret. time, min	%
4	Hexanoic	2.7	6
7	Heptanoic	4.2	9
8	Octanoic	6.0	13
10	Benzoic	7.0	23
16	Salicylic	9.2	5
50	Hexadecanoic	22.2	0.2
53	Heptadecanoic	23.8	0.3
54	Octadecanoic	25.4	0.2

[a] Column: 10 ft × ⅛ in. stainless steel, 2% OV-17 on Gas-Chrom Q, 80–100 mesh; temperature programmed, 60–240°C, 5.6°C/min; detector (FID) temperature, 325°C; inlet temperature, 275°C; flow (He), 25 ml/min.

identification was comprised of a single compound since the symmetry of the peaks did not suggest otherwise.

At least 33 components were evident in the chromatogram of the bases. The compounds identified in Table II account for approximately 85% of the total area of all the peaks observed. Excessive bleeding of the column (28% Pennwalt 223 + 4% KOH on Chromosorb R) at temperatures above 125°C limited the analysis to the more volatile bases. The identities of two of the major peaks, 15 and 16, were tentatively confirmed by GC–MS as pyridine and 2-methylpyridine, respectively. The mass spectrum corresponding to the third major peak, 18, contained major ion abundances at *m/e* 107 and 93 suggesting the presence of a second methylpyridine plus a dimethylpyridine. It was found that both 3-methylpyridine and 4-methylpyridine, as well as 2,6-dimethylpyridine, had retention times identical with peak 18. That all three of the pyridines had identical retention times is less surprising in view of the similarity of their boiling points: 3-methylpyridine, bp 143.5°C; 4-methylpyridine, bp 143.1°C; 2,6-dimethylpyridine, bp 143°C.

Sixty-three peaks were counted in the chromatogram of the silylated phenols, of which the compounds listed in Table III account for approximately 25% of the total peak area. All of the compounds listed have previously been identified in marijuana smoke condensate by GC–MS (Fentiman et al., 1973) with the exception of scopoletin and esculetin. These plant coumarins have been identified in tobacco smoke (Black and Dickens, 1966) and had identical retention times on the column used. While the cresol isomers were poorly resolved and were integrated together, the major portion of peak 8 appeared to be due to *o*- and/or *m*-cresol with a shoulder on the high-temperature side corresponding to *p*-cresol. Since this confirmed the results of previous workers (vide supra), no attempt was made to employ other columns for additional confirmation.

The chromatogram of the methyl esters of smoke condensate acids contained approximately 65 peaks of which the acids listed in Table IV accounted for approximately 57% of the total area. With the exception of salicylic and heptadecanoic acids, all of the acids listed have been previously identified in marijuana smoke by GC–MS (Fentiman et al., 1973), and no other columns were employed for confirmation. Trace amounts of other saturated, straight-chain acids in the C_9–C_{20} range were indicated but not confirmed.

Referring again to Table I, the 16.9% of the total smoke condensate represented by the acids, bases, and phenols is 5 times higher than that found in the ethanolic extraction of *Cannabis* plant material (Groce and Jones, 1973). A previous report describes tobacco pigment, lignin, and pectin as being good phenol precursors (Schlotzhauer et al., 1967) and, previously, it was suggested that 41% of the phenols found in tobacco smoke derived from the carbohydrate content (55%) of flue-cured tobacco (Bell et al., 1966). In a study of the carbohydrate–cyclitol content of *Cannabis* (MS-13 strain), only 6.1% of the plant material extract was found to be comprised of these compounds (Groce and Jones, 1973). Tentative mechanisms can be proposed by which the cyclitols could conceivably dehydrate and demethoxylate under pyrolytic conditions to give phenol, quaicol, catechol, and hydroquinone. The origin of esculetin and/or scopoletin could derive from the pyrolytic degradation of the corresponding glycosides found in various plants (Karrer, 1950) but not previously reported in *Cannabis;* although the acids could be generated by the oxidative pyrolysis of terpenes and the like, only one of the amines (dimethylamine) found could arise from the pyrolytic decarboxylation of an amino acid from proteins. The remainder of the nitrogen-containing compounds require more complex and speculative pathways for generation.

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