Cholesterol in Cigarette Smoke Condensate

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Cholesterol, the principal animal sterol, was found in cigarette smoke condensate both in free and bound form. The cholesterol identification was based on gas-liquid chromatography and mass spectrometry. This sterol accounted for 8.6% of the total sterol content in cigarette smoke condensate, of which about 52% was in the free form and 48% in the bound form. In cigarette tobacco, cholesterol accounted for 10% of the total sterols, of which 48% was in the free form and 52% in bound form. The transfer of free and bound cholesterol from cigarette tobacco to trapped condensate was about 13%, while the total sterol transfer was 15%.

Cigarette smoke condensate is an extremely complex mixture of chemical constituents which includes sterols in free and bound forms. Cholesterol (cholest-5-en-3β-ol), an important animal and plant sterol, has not previously been reported to be present in cigarette smoke. However, this sterol along with campesterol (24α-methylcholest-5-en-3β-ol), β-sitosterol (24β-ethylcholest-5-en-3β-ol), and stigmasterol (24β-ethylcholest-5,22-dien-3β-ol) comprise the major sterols in tobacco leaf (Cook *et al.*, 1967, 1969; Davis *et al.*, 1968; Keller *et al.*, 1969; Richardson *et al.*, 1968; Stedman, 1968). Campesterol, β-sitosterol, and stigmasterol (Grossman and Stedman, 1958; Johnstone and Plimmer, 1959; Kosak *et al.*, 1957) have been identified in cigarette smoke in the free form, and Kallianos *et al.* (1963) found the glycosides of the latter three sterols in cigarette smoke. The fatty acid esters of β-sitosterol and stigmasterol have also been reported in smoke condensate (Rodgman *et al.*, 1959). We wish to report the detection of cholesterol in its free and bound form in cigarette smoke condensate.

EXPERIMENTAL

Chemicals. The sterol reference standards cholesterol, campesterol, stigmasterol, and β-sitosterol were purchased from Applied Science Laboratories, State College, Pa. The β-sitosterol standard contained 95% β-sitosterol and 5% campesterol. Cholestane (Sigma Chemical Co., St. Louis, Mo.) was used as the internal standard. All solvents used were reagent grade.

Instrumentation. The gas-liquid chromatographic system used consisted of an F&M Model 402 Gas Chromatograph equipped with a flame ionization detector, effluent sample splitter, and a Hewlett Packard Model 3370A Electronic Digital Integrator. The column was a 1.80 m U-shaped glass with a 6 mm i.d. and packed with Anakrom ABS 80/90 mesh (Analabs, Hamden, Conn.) coated with 5% OV-101 (Supelco, Bellefonte, Pa.) (Grunwald, 1969). The column temperature was 250° C and the flash heater and flame detector temperatures were kept at least 50° C above that of the column. The carrier gas was helium at a flow rate of 100 ml per min. Ethyl acetate was the solvent in all cases.

The mass measurements were obtained on a RMU-7 Hitachi–Perkin Elmer double focusing mass spectrometer operating at 70 eV with a source draw-out potential of 2.4 kV. The direct inlet temperature at the solid heater was 250° C.

The cigarettes were smoked on a R. W. Mason MK III smoking machine. The smoke was collected in tandem traps, containing 8 mm Rashing rings, submerged in a dry ice-acetone bath.

Cigarette Sample. The University of Kentucky reference cigarette IR1 was used in this investigation. It is an 85 mm nonfiltered cigarette with a pressure drop (H_2O) of 7.6 cm and an average weight of 1.122 g at 11.3% moisture. The cigarettes were purchased from the University of Kentucky Tobacco and Health Research Institute, Lexington, Ky.

Smoke Condensate Preparation. The smoke condensate samples were obtained from 500 cigarettes smoked with 35 ml puffs for 2 sec duration at the rate of one puff per min to a butt length of 23 mm. The smoke condensate in the trap was dissolved in 2000 ml of methanol, and 50 ml of the methanol extract (equivalent to smoke condensate from 12.5 cigarettes) was used for sterol analysis.

Separation of Sterols. The tobacco from 20 cigarettes was ground in a Wiley Mill with a 40-mesh screen and 5 g samples were removed and extracted with 250 ml of acetone in a Soxhlet apparatus for 24 hr.

The total, free, and bound sterols (glycosidic and esterified sterols) were isolated by a modified method of Stedman and Rusaniwskyj (1959). Samples of 50-ml methanol extract of the smoke condensate and the acetone extract of 5 g of tobacco were used for total and free sterol analysis, and values for bound sterols were calculated.

Total sterols were determined on one set of samples by evaporating to dryness under vacuum and adding 25 ml of 95% ethanol containing 0.13 ml of H_2SO_4. The samples were refluxed for 12 hr and at this point 15 ml of 10% KOH in 95% ethanol were added and refluxed for an additional 30 min.

Free sterols were determined on a second set of samples by taking to dryness under vacuum and adding 30 ml of 95% ethanol. The samples were heated for a few minutes and cooled.

From the above mixtures the sterols were extracted three times with 30 ml of *n*-hexane and enough water to obtain two layers. The *n*-hexane fractions of each sample were pooled and back-extracted twice with 90% methanol. The resultant *n*-hexane fractions were taken to dryness and dissolved in 20 ml of boiling absolute ethanol. Added to this mixture were 10 ml of hot 2% digitonin in 80% (w/v) ethanol and 5 ml of hot water. The samples were cooled overnight at room temperature. The resultant sterol-digitonide precipitates were washed three times with 80% ethanol, three times with diethyl ether, and dried overnight at room temperature.

The white sterol-digitonide precipitates were broken with 2.0 ml of pyridine, containing a known amount of cholestane (internal standard), heated at 70° C for 2 hr and left at room temperature for 12 hr. The digitonin was precipitated with 40 ml of diethyl ether and removed by centrifugation. The

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ether layers were taken to dryness and the residue taken up in ethyl acetate for injection into the gas chromatograph. The quantitative sterol analysis was carried out by measuring the peak areas of the sterols and the internal standard, making corrections for the differences in relative weight response of the individual sterols. A more complete discussion of the quantitative procedure has been presented elsewhere (Grunwald, 1970).

The trimethylsilyl (TMS) ethers were formed by dissolving the sterol sample in 0.2 ml of acetonitrile and adding by syringe 0.2 ml of bis(trimethylsilyl)acetamide. The samples were sealed and allowed to react for 1 hr at 30° C (Klebe *et al.*, 1966).

For acetylation, the sterol samples were dissolved in 0.2 ml of pyridine and added were 0.2 ml of acetic anhydride. The samples were allowed to react for 12 hr at room temperature.

RESULTS AND DISCUSSION

The sterol fractions isolated from tobacco and its smoke condensate, when determined as free sterols (Grunwald, 1970), contained four peaks and these corresponded to authentic cholesterol, campesterol, stigmasterol, and β-sitosterol. The relative retention times of the acetates and TMS ethers were the same as those of the authentic sterols and no contaminates could be detected. The glc peak of the smoke condensate corresponding to authentic cholesterol was collected and analyzed with a mass spectrometer. Identified were the following ions specific for cholesterol: *m/e* 386 (100%), 371 (50%), 368 (79%), 358 (68%), 275 (100%), 260 (35%), and 247 (50%). These ions corresponded to *m/e* peaks obtained for authentic cholesterol and those published by Knight (1967).

The quantitative values for total free and bound sterols of tobacco and trapped smoke condensate are given in Table I. The total sterol content per g tobacco was 1606 μg of which 165 μg or 10.3% was cholesterol and the smoke condensate per g tobacco smoked contained 21 μg of cholesterol. Even though the presence of cholesterol in tobacco has been reported (Cook *et al.*, 1967, 1969; Davis *et al.*, 1968; Keller *et al.*, 1969; Richardson *et al.*, 1968; Stedman, 1968), its presence in tobacco smoke condensate had not previously been cited. The transfer of cholesterol from tobacco to its trapped smoke condensate was 12.7%, which was the lowest transfer of the four sterols. The stigmasterol transfer from tobacco to the smoke condensate was 17.9% and was the largest transfer of any of the sterols. Only 15% of the total sterols was trapped in the smoke condensate and the remaining fraction must have been lost in the smoke sidestream, pyrolyzed during the smoking process and/or deposited in the cigarette butt.

The free sterol transfer from the cigarette tobacco to its trapped smoke condensate was 16.7%. Cholesterol transfer was 13.9%, which was the lowest transfer of any free sterol. The transfer of free stigmasterol, campesterol, and β-sitosterol was 16.0, 17.1, and 18.9%, respectively.

The bound sterol fraction includes both the glycosides and the esters. A separate determination for glycosides was not made because they account for only a small portion (about 2%) of the total sterols (Grunwald, 1970a). Bound β-sitosterol transfer at 10.4% was the lowest and bound stigmasterol at 20.2% was the highest sterol transferred. Cholesterol transfer was 11.6%. The transfer of the total bound sterols to the trapped smoke condensate was lower than the transfer of the total free sterols. The generally lower transfer of the bound sterols may have been due to their lower volatility and/or greater pyrolysis during the smoking procedure. However, stigmasterol was an exception—its transfer was 20.2%.

Table I. Total, Free, and Bound Sterol Content of Cigarette Tobacco and Its Trapped Smoke Condensate

Sterol Component	Sterol in Tobacco μg/g dry wt	Sterol in Smoke Condensate μg/g Tobacco Smoked	% Sterol Transferred from Tobacco to Smoke Condensate
	Total Sterols		
Stigmasterol	588	105	17.9
β-sitosterol	551	73	13.2
Campesterol	302	44	14.6
Cholesterol	165	21	12.7
Total	1606	243	15.1
	Free Sterols		
Stigmasterol	326	52	16.0
β-sitosterol	185	35	18.9
Campesterol	129	22	17.1
Cholesterol	79	11	13.9
Total	719	120	16.7
	Bound Sterols		
Stigmasterol	262	53	20.2
β-sitosterol	366	38	10.4
Campesterol	173	22	12.7
Cholesterol	86	10	11.6
Total	887	123	13.9

In conclusion, cholesterol both in free and bound forms was found in smoke condensate, in addition to stigmasterol, β-sitosterol, and campesterol. The bound sterols were transferred to the smoke condensate at a lower rate than the free sterols; however, at the present time it is not known whether the bound sterols were converted to the free form or were decomposed during the smoking process.

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