Alkylating Activity of Cigarette Smoke Condensate

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Biological alkylating agents act by alkylation of essential cellular metabolites in producing toxic, mutagenic and/or tumorigenic effects. Cigarette smoke shows alkylating activity[1,2] but the amount and other details of this property have not been studied. Since the tumorigenic activity of smoke condensate in animals cannot be explained on the basis of its known chemical composition,[3] it is of interest to learn more of the alkylating activity therein.

Smoke condensate from commercial domestic cigarettes was fractionated as described previously.[4,5] All fractions were measured for alkylating activity by a modification of the method of Sawicki *et al.*[2] using 4-(4′-nitrobenzyl)pyridine as the nucleophilic reagent. The modification consisted of using 1:1 (*v*/*v*) acetophenone-diethylene glycol monoethyl ether as the solvent for the condensate and all fractions thereof.

Unfractionated smoke condensate showed the equivalent of 20 micrograms of 2-iodobutane per cigarette in alkylating activity. The distribution of activity is given in the Table. "Specific activity" is a measure of the relative alkylating activity of fractions and is expressed as absorbance per 20 mg. at the absorption maximum (570 mμ). Two values are given for "Strong, water-soluble acids." In the fractionation, this fraction is obtained as an aqueous solution containing 0·1N HCl. During evaporation (*in vacuo* at $< 25°C$) of this solution to a residue, some hydrohalogenation of unsaturated acids probably occurs yielding halogenated acids with alkylating activity as artifacts. Trials with pure crotonic and maleic acids show the development of such activity on evaporation from acidic solutions. In the Table, the analytical value for this fraction is compared with the calculated value obtained by subtracting the total activities of all other fractions (in %) from 100. It should be noted that this fraction should also contain water-soluble, ether-insoluble neutrals.

The bulk of the alkylating activity is found in the cyclohexane-soluble, neutral fraction and in the water-soluble, strongly acidic fraction. The highest specific activity is found in the precipitate ("Ppt A") which forms during fractionation when aqueous hydrogen chloride is added to the ether solution of bases and neutrals. High specific activity is also observed in the smoke pigment obtained when the aqueous extract of acids is reduced in pH from 6·0 to 1·0 ("Strong acid, ppt"). This pigment is related to the high molecular weight polymeric smoke pigment ("Weak acid, ppt") recently shown to contain chlorogenic acid, amino-acids, alkaloids and a silicone.[5,6] The entire pattern of distribution suggests the presence of several alkylating agents in the condensate rather than the distribution of a single agent by differential solubility.

Table

Distribution of alkylating activity in smoke condensate fractions

	Weight %	*Alkylating activity* *Specific*	*Total*
Smoke condensate	100	0·32	100
Neutrals: Cyclohexane-soluble*	29	0·29	26
Nitromethane-soluble*	2·5	0·39	3
MeOH-H_2O-soluble*	3·7	0·57	6·6
Bases: Ether-soluble	6·8	0·18	3·7
H_2O-soluble	1·6	0·44	2·2
Ppt A	0·4	1·1	1·5
Ppt B	0·1	0·70	0·2
Acids: Weak, ether-soluble	11	0·19	6·5
,, ppt.	6·8	0·40	8·5
Strong, ether-soluble	4·2	0·21	2·8
,, ppt.	2·3	0·88	6·5
,, water-soluble (Found)	32	0·84	84
,, ,, ,, (Calc.)	—	0·33	33

*Corrected for small losses in weight ($<6\%$ of total neutrals before sub-fractionation) presumably due to volatility during solvent removal.

The smoke constituents contributing to this activity are not known. Methyl chloride is an alkylating agent in smoke but should not be present in condensate fractions due to its volatility. Epoxides have not been reported in condensate, but the occurrence of an epoxydiol xanthophyll (violazanthin) in green tobacco leaves is known.[7] The presence of epoxides in smoke has been postulated[3] and such compounds may occur in leaf oxidation products.[8] No β-lactones but two epimeric, diterpenoid γ-lactones (α- and β-levantenolides) have been found in smoke.[9] Nitrosamines, which are believed to act as biological alkylating agents,[3] are present in some smoke condensates[10,11] but may be artifacts produced from reactions between oxides of nitrogen and secondary amines during the collection of cigarette smoke at very low temperatures.[12] Contributions to alkylating activity might also be made by such compounds as halogenated hydrocarbons or phosphate esters used as pest control agents during tobacco growth. A possible pyrolysis product of such a hydrocarbon has been reported in smoke.[4]

Since alkylating agents and nucleophiles, *e.g.* pyridine and derivatives thereof,[13] are both present in smoke condensate, an interesting question arises as to why they do not react immediately in aerosol particles or in the condensation traps. A possible answer is that such reactions have occurred and residual activity is being measured. If such is not the case, the observed pattern may be a reflexion of differences in the rates of nucleophilic reactions. For alkylations proceeding by an S_N1 mechanism, rates are related to the dielectric constant of the solvent and cooled (−79°c) condensate may not be favourable in this respect. For S_N2 mechanisms, alkylation is a function of the degree of nucleophilicity of the nucleophile, and the analytical reagent may be more active than the naturally occurring bases.

The chemical determination of alkylating activity provides a useful means of following activity during chemical fractionation. If a correlation between such activity and *in vivo* tumorigenic yields[3] could be demonstrated, studies of the isolation of biologically active compounds from smoke would be facilitated greatly. Studies to determine such a correlation are in progress.

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