TOBACCO ADDITIVES AND CIGARETTE SMOKE

PART I. TRANSFER OF D-GLUCOSE, SUCROSE, AND THEIR DEGRADATION PRODUCTS TO THE SMOKE

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ABSTRACT

Uniformly labeled D-glucose-^{14}C and sucrose-^{14}C added to the burley portion of an American-blend cigarette were largely consumed during smoking. Small proportions (~0.5%) of both sugars were transferred into the smoke unchanged. Even smaller proportions of activity were detected in the 5-(hydroxymethyl)-2-furaldehyde (HMF) and 5-methyl-2-furaldehyde present in the neutral fraction of smoke. Non-volatile acids, bases, and phenols were also formed. An accumulation of HMF and 1,6-anhydro-β-D-glucopyranose was found in the unburned, tobacco stub.

INTRODUCTION

Air-cured tobaccos are characteristically low in reducing sugars. Certain burley tobaccos, used in American-blend cigarettes, yield strong, pungent smokes whose effects are not entirely attributable to alkaloids. Addition of sugars and other casings improves the overall smoking qualities of these air-cured tobaccos[1-8], but few studies of the end-products from the sugars have been made. The fate, on burning, of sugars added to the burley portion of a cigarette blend of tobaccos has therefore been studied.

Bradford *et al.*[3] and Dittmar[4] indicated a lessening of the volatile bases in smoke, and Sugawara *et al.*[5] reported increases in reducing substances in the smoke of cased tobaccos. Others have shown an improvement in the smoking qualities of strong tobaccos by the use of sugars, of organic acids, and of sugars plus glycerol and propylene glycol casings[6-8].

Fagerson[9] and Stedman[10] have reviewed thermal degradations of sugars as related to food and tobacco chemistry. Many products, primarily carbonyl and furan compounds, have been identified by modern instrumental techniques, and mechanisms of formation have been proposed[11-16].

In experiments on cigarette smoke, Quin *et al.*[17] reported that sugars are not significant precursors of the furoic and levulinic acids in smoke. They found that the addition of sugars to all-burley cigarettes did not increase the proportion of volatile acids in the smoke. Larson and Harlow[18] added D-glucose-^{14}C to all-burley cigarettes, and calculated that 7 to 10% of the volatile acids was formed from the D-glucose casing; this range is comparable to the proportion, by weight, of casing normally added[1,2,9]. Other workers, including Bell *et al.*[19], who calculated that 41% of the phenol in smoke arises from carbohydrates, have mechanically smoked radioactive cigarettes, but no-one has added radioactive sugars to the burley portion of a blend and then examined the smoke[20–24].

Sugars are both additives and natural constituents of commercial tobacco products. As they are nonvolatile and undergo change on heating above the melting point, they are not added as flavorants *per se*, as with menthol, but for an overall effect. As a part of our studies on the chemistry of cigarette smoke (including an understanding of the mechanisms of transfer of compounds to smoke and, specifically, the determination of the fate of sugar casings), experimental cigarettes containing radioactive sugars were made and mechanically smoked. The complexity of the chemistry involved is indicated by the distribution of activity in the various fractions of the smoke of cigarettes to which uniformly labeled sugars had been added to the burley tobacco *only*, rather than to the entire blend.

RESULTS AND DISCUSSION

The mainstream (intermittent puffs) and sidestream (continuous smolder in air) of cigarette smoke consist of mixtures of gases and particulate matter. Test cigarettes containing radioactive sugars were smoked, and the distribution of activity was determined in the gas phase and in the total particulate matter (TPM) of both streams, in the unburned tobacco (butt or stub), and in the ash. The gross distribution shown in Table I is representative of the data obtained. Material-balance data for

TABLE I

GROSS DISTRIBUTION OF RADIOACTIVITY, IN PERCENTAGES OF TOTAL ACTIVITY

	D-*Glucose*-^{14}C			*Sucrose*-^{14}C		
Mainstream						
gas phase						
CO_2	2.7			3.1		
others	2.0			2.8		
		4.7			5.9	
TPM		1.7			3.0	
			6.4			8.9
Sidestream						
gas phase						
CO_2	37.4			41.8		
others	8.4			9.8		
		45.8			51.6	
TPM		3.7			3.8	
washes		1.5			1.4	
			51.0			56.8
Butt			41.0			40.6
Ash			1.5			1.7
Recovery (%)			99.9			108.0

five such experiments gave an average of 100.3%, with a standard deviation of ±10.9%. Uneven distribution of the tobacco in the blend, as well as within individual cigarettes, presumably explains some of the deviation. For instance, a low radioanalysis combined with a low weight for the butt of one cigarette resulted in our lowest "recovery" (82%).

As expected for nonvolatile materials, the largest proportions of the radioactivity were found in the sidestream gases. About 40% of the total activity was in the carbon dioxide, and another 10% was incompletely combusted to carbon monoxide and organic products.

Small proportions of radioactivity accumulated in the butts (3.5%) and ash (1.5%). Paper-chromatographic analyses indicated the presence of 1,6-anhydro-β-D-glucopyranose (levoglucosan) and 5-(hydroxymethyl)-2-furaldehyde (HMF) in ether extracts of the butts. The extracts contained 0.5% of the initial activity, with two-thirds of it in the HMF zone. The levoglucosan was estimated to represent 0.05% of the sugars added.

In the mainstream, up to 3% of the total activity was present in the TPM, but this was less than half of the combined gaseous components. For D-glucose, the level was lower than that found by Thornton and Valentine[22] in the mainstream TPM (1.8, compared to 4.9%), and higher for the sidestream gases (45.8, as against 26.8%). The methods of adding the sugars were different, as were the cigarettes (hand-made *versus* commercial) and the tobaccos used, so that further comparisons cannot be made.

A further analysis of the distributions of activity within the mainstream fractions is presented in Table II. Here, too, carbon dioxide is the major single component. The mass ratio of total carbon dioxide to combustibles was lower (5:3 *versus* 3:1) than for the sidestream. The specific activities of the mainstream carbon dioxide and "CO" (see later) and of the sidestream combustibles were comparable, but that of the carbon dioxide in the sidestream was higher (1.4:1) for both sugars. The gross data for mass and activity show the increased combustion occurring during the intervals between puffs, but the increased specific activity is probably a result of the fact that the sugars were on the surface of the tobacco.

The "CO" fraction of the mainstream was not analyzed qualitatively, but presumably contained the carbon monoxide plus methane, ethane, and other components not retained by a silicone column at −68°. Subsequent elution of the column showed no retention of carbon dioxide. The analysis of the organic constituents retained is reported[25] in Part II.

Each of the sugars was found in the aqueous extracts of the particulate phase. The levels of about 0.5% (range 0.4 to 0.8%) are considerable, although much smaller than those for carbon dioxide and "CO". The presence of these nonvolatile constituents which decompose on melting is indicative of a mechanical carry-over into the smoke. Such an entrainment presumably explains the presence of amino acids, sterols, and pigments of high molecular weight found by others[10]. Cogbill and Hobbs[26] concluded that some metallic constituents are transferred to smoke by such

TABLE II

DISTRIBUTION OF RADIOACTIVITY IN MAINSTREAM SMOKE, IN PERCENTAGES OF TOTAL ACTIVITY

Smoke	D-*Glucose*-^{14}C			*Sucrose*-^{14}C		
I. Gas phase						
CO_2	2.7			3.1		
CO	1.7			2.4		
organic compounds	0.3			0.4		
			4.7			5.9
II. TPM						
vacuum distillate						
nonpolar	0.21			0.19		
polar	0.12			0.10		
		0.33			0.29	
nonvolatile						
nonpolar						
bases	0.11			0.21		
acids	0.06			0.10		
neutrals	0.18			0.30		
phenolics	0.04			0.11		
		0.39			0.72	
polar		0.92			1.28	
pads and washes		0.06			0.03	
			1.7			2.3
			6.4			8.2

a mechanism, but the levels (0.001 to 0.003%) were considerably lower. Because we added the sugars to the surface, the entrainment and combustion yields might be expected to be higher than for other constituents naturally present. Sugars have been identified in cigarette smoke[27]. The levels for total reducing sugars from burley and bright tobaccos, respectively, were 75 and 560 μg per cigarette, expressed as glucose.

In preliminary experiments, poor recoveries were obtained when fractionation of the TPM was attempted by extractions and ion-exchange chromatography. Activity was, however, found in the acid, base, and neutral fractions of the TPM. To prevent the loss of volatile materials in the TPM, the mainstream pads were sealed, and distilled under vacuum as soon as possible. "Nonvolatile" fractions were obtained, with overall recoveries as high as 99% of the activity initially found in the ether–water partition. The presence of 5-(hydroxymethyl)-2-furaldehyde and 5-methyl-2-furaldehyde in the neutral fraction was determined by paper or gas chromatography, or both. Chromatographic fractions, eluted with or collected in methanol, were radioactive. Quantitative estimations by u.v. spectroscopy were possible, but background absorption indicated impurity, even after the use of three chromatographic systems in the case of HMF. Whether these furaldehydes distil after initial dehydration of the sugars, or are products of the degradation of initially formed polymers as predicted by Gardiner[11], has not yet been established.

EXPERIMENTAL

Cigarettes and smoking conditions. — The burley portion of an experimental blend of cigarette (cigt.) tobaccos was sprayed with an alcoholic solution of D-glucose-^{14}C (U) (5.4 mCi/mmole, Merck and Co., Ltd.) or sucrose-^{14}C(U) (15 mCi/mmole, Nuclear-Chicago), each >98% radiochemically pure. Cigarettes (67 mm long; 1.085 ±0.030 g, 29 μCi of D-glucose-^{14}C/cigt.; 1.065 ±0.040 g, 7 μCi of sucrose-^{14}C/cigt.) were made by hand from the complete blend and Excel cigarette-paper tubes; they were equilibrated at 75°F and 60% relative humidity before being smoked. One, two, or three cigarettes were smoked to a 25-mm length of butt with a closed, smoke-collection system. Smoke was generated by a cryogenic puffing system consisting of a timed solenoid valve and an adjustable orifice (Teflon-and-glass needle-valve) with the vacuum supplied by the condensation of gases on charcoal at the temperature of boiling liquid nitrogen. The mainstream particulate phase was collected on a Cambridge filter, and the vapors were passed through the solenoid and orifice into two gas traps in series; the first contained glass wool, and the second, charcoal, both cooled by liquid nitrogen. The orifice was adjusted to give a 35-ml puff each time the solenoid opened (two seconds duration, once a minute). The sidestream smoke was collected similarly, but the solenoid valve was omitted, and the orifice was adjusted to give a continuous air-flow of 550 ml/min supplemented by nitrogen at 50 ml/min. At the conclusion of smoking, nitrogen at 550 ml/min replaced the air and extinguished the cigarette within 20 sec. An additional "clearing puff" was drawn through the system in order to collect any residual smoke. Commercial cigarettes gave puff counts and deliveries of nicotine and TPM comparable to those obtained with multiport, syringe machines.

Analyses. — At the completion of the smoke collection, solids were weighed and the glassware was rinsed. The butts were successively extracted with ether and water, and the carbon in the residues and in the ash was converted into carbon dioxide by wet oxidation.

The TPM for both streams was kept sealed until it was analyzed. The mainstream pad was distilled at room temperature/100 mtorr. The condensate, collected at the boiling temperature of liquid nitrogen, was partitioned between ether and water, and each layer was assayed for radioactivity. The "nonvolatiles" were eluted from the pad with methanol, ether, and water. The methanol was evaporated, and the residue was combined with the other extracts for partitioning. Aliquots of the initial layers were assayed for activity. The rest was used for obtaining acidic, basic, neutral, and phenolic fractions by a series of extractions with ether at appropriate pH values. The polar materials were examined by paper chromatography and/or ion-exchange paper chromatography and column chromatography.

The sidestream TPM was extracted from the pad with methanol and water, and the extracts were assayed. The colored residue remaining on the pad was oxidized to carbon dioxide prior to counting.

The mainstream gases in the trap containing glass wool were transferred to a precooled (Dry Ice), gas-chromatographic column containing 0.95 g of 5% QF-1 on Gas Chrom Z. Gases not retained by the column passed through a bubbler into 40% potassium hydroxide, and then through a conventional combustion-furnace containing cupric oxide, and another potassium hydroxide trap. Helium (60 ml/min) was used for supplementing the flow of the components as they thawed and distilled from the trap while it warmed up. The trap was then heated for 20 min with boiling water. The gases from the other traps were passed separately through the potassium hydroxide traps and the furnace, but not through the cold column. The carbon dioxide from the smoke, together with that arising from the combustion of vapors, was precipitated as barium carbonate, which was dried and weighed, and its radioactivity counted.

Radioactivity was determined in liquid samples by scintillation counting. Organic solids were wet-combusted to carbon dioxide; barium carbonate precipitates were converted into carbon dioxide by means of lactic acid, and the radioactivity of the gas was counted in ion chambers. Paper chromatograms were monitored with a recording Scanogram.

The solvent systems used were: 8:1:1 butanone–acetic acid–water saturated with boric acid, 15:3:2 ethyl acetate–pyridine–water, 7:3 chloroform–95% ethanol, and 6:7:3 benzene–acetic acid–water. Deionized water was used with Whatman resin-impregnated papers (SA-2 and WB-2) and with mixed-bed, ion-exchange resins [IR-120 (H^+) and IRA-4B (OH^-)].

The gas-chromatographic conditions were: glass column (4 ft × 0.25 in. o.d.) containing 5.3 g of 15% Carbowax 20M on Chromosorb W (80–100 mesh); 160°; argon ionization detector; argon flow of 30 ml/min.

Solvent blanks were carried through the chromatographic procedures for ultraviolet or scintillation analyses.

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