The Use of Non-Volatile Adducts in Smoke Flavor [1]

Ernest W. Robb, John J. Westbrook III and A. Bavley
Philip Morris Research Center, Richmond, Virginia, U.S.A.

Flavors for smoking products are conveniently added directly to the tobacco at some stage in the manufacturing process. If the flavoring material is volatile, much of it may be lost by evaporation during subsequent manufacturing steps and storage of the finished product. The flavorant may also contribute undesirably to the pack aroma of the product. Evaporation losses are appreciable with flavors presently in use such as menthol and become more acute with experimental flavors of greater volatility. We encountered this problem while studying the effect on total smoke flavor of smoke constituents such as isoprene, furan, or acetaldehyde. These cannot be added to tobacco since they evaporate before cigarettes can be made.

Several proposals have been made for reducing the volatility of tobacco flavors without impairing their release into smoke. Adsorption on bentonite, silica gel, alumina, or charcoal has been suggested (1). Jones (2) and Teague (3) have introduced flavorful acids into smoke by adding non-volatile ester derivatives to tobacco. This method is applicable only when the desired flavorant is an acid.

In this work the use of non-volatile adducts of volatile flavorants was investigated. Useful adducts must be non-volatile, odorless, and stable under conditions of storage on tobacco. They must decompose thermally to regenerate the flavor in good yield and any other products formed must either be non-volatile or odorless and physiologically harmless. Two types of adducts have been found which satisfy these requirements: the adducts formed in the Diels-Alder reaction and the inclusion compounds formed by the cyclodextrins.

The cyclodextrins or Schardinger dextrins are carbohydrates formed by enzymatic degradation of starch. *Alpha-*, *beta,* and *gamma*-cyclodextrin are cyclic compounds in which six, seven, and eight glucose molecules, respectively, are linked in a large ring by *beta*-glucoside linkages. The adducts which they form with organic compounds are believed to be inclusion compounds or clathrates. Recent reviews on the cyclodextrins (4) and on inclusion compound formation (5, 6) are available.

Experimental

Diels-Alder adducts: The adducts shown in **Table 1** were prepared by the published procedures (7-15). Sodium salts were made by adding 2N sodium hydroxide to a stirred suspension of the adduct (2.0 g.) in water (50 ml.) at 65° C. until a permanent phenolphthalein end point was reached. The sodium salts, obtained by evaporating the resulting solutions, were all white solids of indefinite melting point, soluble in water or ethanol, and insoluble in non-polar solvents.

Cyclodextrin adducts: Alpha-, beta-, and gamma-cyclodextrins were separated from a crude cyclodextrin mixture[2] and purified by the published procedure (4). The adducts were prepared by adding 2.0 ml. of the flavorant to an aqueous cyclodextrin solution (*alpha*-cyclodextrin 1.0 g. in 10 ml., *beta* cyclodextrin 0.5 g. in 20 ml., *gamma*-cyclodextrin 0.3 g. in 10 ml.) and stirring the mixture for 1-4 hours. The precipitate of adduct was filtered, dried in air, and washed first with absolute ethanol, then with anhydrous ether until it was odorless.

All of the adducts prepared were white solids, insoluble in water and organic solvents. They were decomposed rapidly by boiling water into the original components. All of them decomposed without melting at 290-310° C. resulting in volatilization of the guest component accompanied by charring of the cyclodextrin residue.

Analysis of the cyclodextrin adducts: A suspension of the adduct (0.100 g.) in 5.0 ml. of water was brought to the boil and quickly extracted with 2.0 ml. of iso-octane. A 10 μl. aliquot of the extract was chromatographed on a 2 m. column R, using a Perkin-Elmer model 154 Vapor Fractometer. The column temperature and flow rate were varied depending on the compound under investigation. The amount of flavorant was estimated by comparing its peak height with a plot of peak height versus concentration for the particular compound. Heating and extracting the cyclodextrin solution a second time did not yield any additional guest compound. In some cases the amount of flavorant in the iso-octane extract was determined by infrared or ultra-violet spectroscopy.

Stability of the cyclodextrin adduct on tobacco: A sample of the menthol-*beta*-cyclodextrin adduct (6.73 g.) which analyzed 6.15% menthol was finely ground and intimately mixed with 135 g. of tobacco. The mixture was stored in a dessicator over saturated ammonium chloride (relative humidity 79%) for three months. The adduct was then mechanically separated from the tobacco, washed with ether, and analyzed. The menthol content, 5.82%, indicated that the adduct was 95% undecomposed.

Pyrolysis products from sodium maleate: The sodium salt (0.50 g.) of the adduct from menthofuran and maleic anhydride was heated in a distilling flask by a bath of Woods' metal at 450°C. for 10 minutes. The receiving flask led to a trap cooled in liquid nitrogen. The infrared spectrum of the material in the receiving

Table 1. Pyrolysis of Diels-Alder Adducts

Adduct	m.p. (lit. m.p.)	ref.	Pyrolysis Products at 750°C (yield)
*Myrcene-maleic acid	121-123° (123.5°)	7	Myrcene (32%)
*Anethole-maleic anhydride	246-248° (249°)	8	Anethole (14%)
*Alpha-phellandrene-maleic anhydride	127-128° (126°)	9	alpha-Phellandrene (8%) p-Cymene (32%)
*Menthofuran-maleic anhydride	131-133° (138°)	10	Menthofuran (30%)
*Isoprene-maleic anhydride	63-64° (64°)	11	Isoprene (37%) Dipentene (2%)
*Cyclopentadiene-acetylenedicarboxylic acid	168-170° (170°)	12	Cyclopentadiene (26%)
*Furan-acetylenedicarboxylic acid	154-156° (158°)	13	Furan (33%)
endo-Dehydronorborneol	106-108° (111°)	14	Cyclopentadiene (17%) Acetaldehyde (12%) Benzene (1%)
Isoprene-cinnamaldehyde	b.p. 89-93°/1 mm. (154-155°/10 mm.)	15	Isoprene (18%) Cinnamaldehyde (13%) Benzene (2%) Toluene (1%) Styrene (ca. 0.1%) Dipentene (ca. 0.1%) 2-Phenyl-4-methylbenzaldehyde (ca. 0.01%)

*Converted to sodium salt before pyrolysis.

flask (0.21 g.) was the same as that of menthofuran. The material in the cold trap, identified by mass spectrometric analysis, consisted of carbon dioxide containing 1.1 mole percent acetylene. The residue in the distilling flask (0.15 g.) was extracted with boiling water, giving a strongly alkaline solution which liberated carbon dioxide on acidification and a black residue (0.05 g.) which was insoluble in all solvents. A pyrolysis of disodium maleate carried out in the same manner likewise yielded carbon dioxide, traces of acetylene, and a residue having similar properties.

Pyrolysis of Diels-Alder adducts: The pyrolysis reaction vessel was a stainless steel tube, 60 mm. by 5 mm. i.d., heated by resistance. A thermocouple inside the tube measured an equilibrium temperature of 750°C. within 10 seconds after the start of the heating. The sample, mixed with three times its weight of Celite, was packed in the tube and a stream of air was passed through the tube at a rate of 15-25 ml. per minute. The air stream then passed through a trap cooled in liquid nitrogen. The total pyrolysis time was 60 seconds at 750°C.

The pyrolysate in the liquid nitrogen trap, after warming to room temperature, was dissolved in hexane or acetone and examined by gas chromatography, using the chromatographic equipment and conditions described above. Compounds were identified by comparing their retention times with those of reference compounds. In addition, the components were trapped from the chromatograph and their identification was confirmed by comparing their infrared and ultraviolet spectra with those of reference compounds. Yields were estimated by comparing chromatographic peak heights with those from known quantities of the respective reference materials.

In one case, in the chromatogram of the pyrolysate from the cinnamaldehyde—isoprene adduct, a peak which did not correspond to any available reference compound was observed. The spectral properties of the compound (infrared bands at 3.65, 5 95, 6.25, 6.70, 6.92, 7.25, 7.95, 12.5, 13.4 and 14.3 microns; ultraviolet maxima at 243, 250, and 290 millimicrons) were consistent with its proposed formulation as 2-phenyl-4-methylbenzaldehyde. This compound, previously unreported, is a dehydrogenation product of the starting adduct.

Pyrolysis of the cyclodextrins and their adducts: The same pyrolysis apparatus and procedure were used.

Each pyrolysate was examined by mass spectrometric analysis and by capillary column gas chromatography. The samples were chromatographed simultaneously at room temperature on a 300 foot squalane column and a 100 foot 2-phenoxyethanol column connected by means of a sample splitter, each column having a flame gauge detector. A complex pattern of 89 distinct peaks was obtained from the cyclodextrin pyrolysate on the two columns. Chromatography of the pyrolysates from the cyclodextrin adducts gave identical patterns except that on each pattern was superimposed a much larger peak whose retention times correspond to those of the flavorant used in forming the adduct.

In the cyclodextrin pyrolysate the following compounds were identified by mass spectrometric analysis: methanol, ethylene, acetaldehyde, acetone, acrolein, 3-methyl-1-butene, furan, and 2-methylfuran. In addition to these, the following compounds were provisionally identified by the correspondence of retention times on the two columns with those of known compounds: methane, ethane, propane, propene, propionaldehyde, 1-butene, 2-methylbutene-1, n-butane, butadiene, 2,3-butanedione, 2-butanone, pentane, and 1-hexane.

Preparation of cigarettes and smoking procedure: Ethanolic solutions of the Diels-Alder adducts or their sodium salts were sprayed onto tobacco. The cyclodextrin adducts were applied by tumbling tobacco, moistened with glycerol, with the powdered adduct. The amount of adduct applied was in the range 1.0-3.0 g. per 100 g. tobacco. The treated tobacco was made into 70

Table 2. Yield of Adducts with Beta-Cyclodextrin

Class	Compound	% Yield	Class	Compound	% Yield
hydrocarbons	n-pentane	0	ketones, cont.	2-acetylpyrrole	0
	n-hexane	0		methylheptenone	71
	n-heptane	12		menthone	90
	n-octane	81	aldehydes	propionaldehyde	0
	n-decane	92		furfural	62
	decalin	42		benzaldehyde	57
	benzene	82		cinnamaldehyde	39
	toluene	86		piperonal	25
	dipentene	98		vanillin	0
	myrcene	54		ethyl vanillin	38
	alpha-pinene	80		citronellal	82
ethers	diethyl ether	0	phenols	phenol	0
	furan	90		p-cresol	74
	cineol	78		m-cresol	74
alcohols	ethanol	0		o-cresol	76
	benzyl alcohol	58		2,4-xylenol	88
	phenethyl alcohol	73		3,5-xylenol	84
	menthol	80-90	acids	formic acid	0
	geraniol	94		acetic acid	0
	linalool	82		propionic acid	33
esters	ethyl acetate	0		butyric acid	50
	coumarin	54		valeric acid	77
	methyl salicylate	90		C_6-C_{20}	85-100
ketones	methyl isobutyl ketone	0			
	cyclohexanone	0			

mm. unfiltered cigarettes.

For quantitative determination of the added flavors in smoke, the cigarettes were mechanically smoked by a standard procedure (16), taking a two second, 35 ml. puff once a minute. Cambridge filter pads were used to separate the gaseous and particulate phases. Furan was determined in the gas phase by a mass spectrometric method. Anethole was analyzed by differential ultraviolet spectrophotometry, measuring an ethanolic extract of the filter pads against a similar extract from a control cigarette, using the peak at 260 millimicrons. Menthol was determined by a standard gas chromatographic method.

Other flavorants were not determined quantitatively. They were qualitatively detected in smoke and their effect on smoke flavor was evaluated by a subjective panel. These flavorants included menthofuran, cinnamaldehyde, linalool, geraniol, cineol, citronellal, phenylethyl alcohol, and coumarin.

Gas chromatograhpy of cigarette smoke: The cigarettes were manually smoked, taking a 35 ml. puff of approximately 2 seconds duration each minute. The gas phase portion of the fourth puff was chromatographed on the squalane and phenoxyethanol capillary columns described above. The complex chromatographic pattern (84 peaks) obtained from a

cigarette containing 30 mg. of *alpha*-cyclodextrin was very similar to the pattern obtained from a control cigarette. No peaks were present which were not also observed in the control pattern, and there were only small differences in peak heights. A cigarette which contained 30 mg. of the *alpha*-cyclodextrin-furan adduct gave an identical chromatographic pattern except that the peak whose retention time corresponded to that of known furan was greatly increased.

Results and Discussion

To establish the utility of Diels-Alder and cyclodextrin adducts for adding flavors to cigarette smoke, it was necessary to determine that appropriate adducts could be prepared readily, that they were stable on storage, that they released the flavorant on heating, that this release occurred in burning cigarettes, and that the non-flavorant portion of the adduct was not likely to add undesired constituents to the smoke.

A number of Diels-Alder adducts in which either the diene or dienophile moiety was of interest for smoke flavor has been described. Those studied in this work are listed in **Table 1**.

Since the presence of either maleic anhydride or acetylene-dicarboxylic acid in smoke would be undesirable, the adducts from these dienophiles

were reacted with alkali, to open the anhydride rings and neutralize the carboxyl groups formed. The resulting sodium salts were used in subsequent experiments. A reverse Diels-Alder reaction of these salts would be expected to yield the diene and either disodium maleate or disodium acetylene-dicarboxylate. When the sodium salt of the menthofuran-maleic anhydride adduct was heated at 450°C., there was formed, in addition to menthofuran, carbon dioxide, sodium carbonate, a trace of acetylene, and a carbonaceous residue. These products were assumed to arise from thermal decomposition of the disodium maleate initially formed, since pyrolysis of disodium maleate gave a similar mixture of products.

Cyclodextrin adducts were readily prepared from a wide range of compounds. In **Table 2** are listed the yields of adducts obtained from *beta*-cyclodextrin and a number of potential flavorants. The chief limitations on adduct formation are that acyclic compounds having a chain length of approximately seven or less do not form adducts and that water soluble compounds do not form adducts under our conditions.

Adduct formation is not limited to pure compounds. Adducts were obtained in 50-70% yield from oil of peach, oil of orange, and oil of lemon. When these adducts were decomposed

(Tobacco Science 5)

by boiling water the odors released were indistinguishable from those of the original oils. Evidently those compounds in the natural mixture most responsible for its odor are efficiently included by the cyclodextrin.

The cyclodextrin adducts contain 3% to 12% of the included component. Some analyses are shown in **Table 3**. The molar ratio of included component to cyclodextrin was generally less than unity.

Since these adducts are decomposed rapidly by hot water, it was necessary to demonstrate that they were stable on tobacco. This was done by storing a mixture of the menthol-cyclodextrin adduct and tobacco at a high moisture level. The adduct was found to be 95% undecomposed after three months.

The release of the flavorant portion of the adducts upon heating was demonstrated by pyrolyzing the adducts at 750°C. in a stream of air, a procedure designed to approximate conditions occurring in a burning cigarette. The pyrolytic products from the Diels-Alder adducts are given in **Table 1** along with their yields, which are reported as the percent of the amount theoretically expected from the reaction stoichiometry. The majority of the adducts decomposed cleanly yielding only those products expected from a reverse Diels-Alder reaction. Dehydronorborneol, formally a Diels-Alder adduct from cyclopentadiene and vinyl alcohol, yielded cyclopentadiene and acetaldehyde, showing that its thermal decomposition occurs as a reverse Diels-Alder reaction.

Exceptions to this were the *alpha*-phellandrene-maleic anhydride adduct, where dehydrogenation of the initial product to *p*-cymene occurred, and the cinnamaldehyde-isoprene adduct, where in addition to the expected products, compounds arising

from more extensive decomposition were observed. In cases where isoprene was formed it was accompanied by a smaller quantity of its dimer, dipentene.

The cyclodextrin adducts lose their included molecule when they are heated to about 300°C., the decomposition point of the cyclodextrin. Pyrolysis of these adducts in air at 750°C. caused volatilization of the included flavorant and extensive decomposition of the cyclodextrin. A number of decomposition products were tentatively identified by mass-spectrometry and gas chromatography (see Experimental Section). Most of the compounds thus identified have been found previously in pyrolysates from other carbohydrates (17, 18) and are also normal constituents of cigarette smoke (19).

The thermal release of flavorants from the adducts occurred during smoking when the adducts were applied to tobacco in cigarettes. The odor of some flavorants was distinctive enough that they could readily be detected subjectively in cigarette smoke. In these cases smoking by panel members was sufficient to establish the presence of the flavorant. In some other cases the amount of flavorant added to the smoke was measured analytically. These data are shown in **Table 4**. The yields are calculated as the amount found in smoke divided by the amount added to the tobacco in the form of adduct. The yields are not high, however only 15-25% of an additive such as menthol applied directly to tobacco is found in mainstream smoke, since a major portion of the tobacco in a cigarette is burned between puffs or is left unconsumed in the butt.

The addition of undesired decomposition products from these adducts to cigarette smoke appears unlikely. The clean pyrolysis observed in the

Table 3. Analyses of Cyclodextrin Adducts

Guest	Cyclodextrin	% Guest in Adduct	Method of Analysis	molar ratio; Guest to cyclodextrin
anethole	alpha	5.2	UV	.36
benzene	alpha	3.1	UV	.39
d-limonene	alpha	3.1	GLC	.23
d-limonene	beta	4.7	GLC	.41
d-limonene	gamma	6.6	GLC	.67
l-menthol	alpha	3.6	GLC,IR	.23
l-menthol	beta	6.4	GLC,IR	.49
l-menthol	gamma	11.8	GLC	1.11
p-cresol	alpha	11.3	UV	1.15
p-cresol	beta	4.2	UV	.46
m-cresol	beta	2.8	UV	.55
o-cresol	beta	5.0	UV	.30
2,4-xylenol	beta	4.5	UV	.43
3,5-xylenol	beta	3.2	UV	.30

Table 4. Yields of Flavorant in Cigarette Smoke

Adduct	% Flavorant in Adduct	Level Adduct Applied (g. per 100 g. tobacco)	Flavorant in Smoke (mg. per cigarette)	Yield Per Cent
alpha-cyclodextrin menthol	3.6%[b]	1.63	.05	8.5%
beta-cyclodextrin menthol	6.4%[b]	2.50	.14	8.8%
anethole-maleic anhydride[a]	31.6%[c]	0.75	.12	5.1%
furan-acetylene dicarboxylic acid[a]	46.4%[c]	2.00	1.08	11.6%

[a] applied as the sodium salt.
[b] Analytical value.
[c] calculated from empirical formula.

Diels-Alder adducts can be assumed to occur also in cigarettes. The pyrolysis products from the cyclodextrins were shown to be similar in nature to those from other glucose polymers which occur abundantly in tobacco. As additional evidence on this point we cite cigarettes containing either the furan-acetylenedicarboxylic acid adduct or the furan-*alpha*-cyclodextrin adduct. The mass spectrometric analysis of the gas-phase smoke from the former cigarettes revealed that, with the exception of furan, the amounts of the 20 gas phase constituents analyzed by this method were not different from those found from control cigarettes. Likewise, gas chromatograms of the gas-phase smoke from the latter cigarettes on two capillary columns, when compared with chromatograms from control cigarettes, did not show any evidence of additional compounds exogenous to normal cigarette smoke. In no case were undesirable flavor notes observed during subjective smoking evaluation of additive-containing cigarettes.

The use of Diels-Alder adducts in studying smoke flavor is limited to flavorants which can act as either dienes or dienophiles. The cyclodextrin adducts have no such limitation. However, due to the small percentage of flavorant in the cyclodextrin adduct, there is a practical limitation on the amount of flavorant which can be added by this method.

Acknowledgements

Literature Cited

1. R. P. Allen, U. S. Patent 2,063,-014, December 8, 1936.
2. S. O'B. Jones, U. S. Patent 2,766,145, October 9, 1956.
3. C. E. Teague, Jr., U. S. Patent 2,766,150, October 9, 1956.
4. D. French *The Schardinger Dextrins*, in Advances in Carbohydrate Chemistry, ed. by M. L. Wolfrom, Vol. *12* (1957), pp. 190-261. Academic Press, New York, 1957.
5. L. Mandelcorn, *Chem. Reviews* 59: 827 (1959).
6. F. Cramer, *Die Einschlussverbindungen*, Springer-Verlag, Berlin, 1954.
7. L. A. Goldblatt and S. Palkin, *J. Amer. Chem. Soc.* 63: 3517 (1941)
8. R. C. Cookson and N. S. Warinyar, *J. Chem. Soc.* 2302 (1956).
9. N. F. Goodway and T. F. West, *J. Soc. Chem. Ind.* 56: 472T (1937).
10. W. Treibs, *Ber.* 70B: 85 (1937).
11. J. Boeseken and W. J. F. de R. van Gracht, *Rec. Trav. Chem.* 56: 1203 (1937).
12. O. Diels and K. Alder, *Ann.*, 490: 236 (1931).
13. O. Diels and K. Alder, *Ann.*, 490: 243 (1932).
14. J. D. Roberts, E. R. Trumbull, Jr., W. Bennett, and R. Armstrong, *J. Amer. Chem. Soc.* 72: 3116(1950).
15. N. P. Sopov and M. L. Kovner, *Zhur Obschei Khim* 28: 2145 (1958)
16. A. O'Keeffe and R. C. Lieser, *Tobacco Science*, 2: 73 (1958).
17. C. T. Greenwood, J. H. Knox, and E Milne, *Chemistry and Industry*, 1878 (1961)
18. S. B. Martin and R. W. Ranstad, *Anal. Chem.* 33: 982 (1961).
19. R. A. W. Johnstone and J. R. Plimmer, *Chem. Rev.* 59: 885 (1951).

———✻———