Effluent from the column passed into a single-stage, glass, jet molecular separator (275 °C) and then into the ion source of a Finnigan Model 3300 mass spectrometer operated in the electron impact ionization mode. The mass spectrum was scanned every 4 s, and the data were acquired, stored, and manipulated by using a Finnigan Model 6110 data acquisition system. Compound peak areas were obtained by using standard software supplied by the manufacturer and were used to estimate products yields.

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On the Fate of Maleic Hydrazide in Tobacco Smokes

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Tobacco main-stream (MSS) and side-stream (SSS) smokes, butts, and ashes from commercial cigarettes and cigarettes made from tobacco treatments containing (1) maleic hydrazide (MH), (2) MH-30 (MH diethanolamine salt, DEA-MH), (3) Royal MH-30 (K-MH), (4) 1R1, and (5) 1968 and 1980 commercial cigarettes were analyzed for their MH contents. The MH transfer rates obtained for MSS ranged from 5.54% for MH-30 to 1.25% for K-MH and for SSS ranged from 3.33% for MH-30 to undetected for MH (acid forms). Further, analysis of MH in butts and ashes along with that in MSS and SSS indicates that there was greater MH destruction when MH in nonvolatile form was used. These results suggest the existence of the possibility of reducing the MH transfer rates in MSS and SSS by using the appropriate form of MH.

Like all commercial crops, various chemicals are also applied to tobacco. One such chemical, maleic hydrazide (MH), has been found to be the most effective substance known so far in tobacco sucker control (Seltmann, 1971). However, this compound is suspected to be a carcinogen (Epstein et al., 1967), and the German government has set a tolerance level of 80 ppm of this substance in cigarette tobacco (Spears and Jones, 1981). This would adversely affect the U.S. tobacco exports, since recent surveys have shown that a sizable amount of U.S. tobacco contains more then 80 ppm of MH residues (Hayes, 1979; Davis et al., 1979; Hunt et al., 1977; Spears and Jones, 1981).

However, as far as the smoker is concerned, the most important question is not how much pesticide is in tobacco but how much of the pesticide and its degradation products is present in cigarette main-stream smoke? Our investigations are, in part, an answer to that and, especially, the MH question.

In our investigations we have analyzed cigarette tobacco, cigarette main-stream and side-stream smokes, cigarette butts, and cigarette ashes for their MH contents and have calculated the transfer of MH into main-stream and side-stream smokes. Since it is a well-known fact that different formulations of the same amount of a pesticide will leave different amounts of pesticide residues in crops, we have also included in our study cigarettes made from tobacco treated with different forms of MH: i.e., MH, acid form [$C_4H_4N_2O_2$ (acid-MH)], Royal MH-30 (potassium salt of MH, K-MH), and MH-30 (diethanolamine salt of MH, DEA-MH). In this respect, as far as we could ascertain, ours is the first such study on any pesticide in tobacco smokes.

Also, although there have been some studies published on the transfer of pesticide residues into the main-stream smoke (Atallah and Dorough, 1975; Guthrie, 1968; Haeberer and Chortyk, 1974; Hengy and Thirion, 1970, 1971; Hoffmann and Rathkamp, 1968; Liu and Hoffmann, 1973), we have come across only one publication (Atallah and Dorough, 1975) dealing with the transfer of pesticide residues into the side-stream smoke. This study does not deal with MH. Further, in this study pesticides were applied to cigarettes by injecting their solutions into the cigarettes. In such application, according to the same authors, the concentration of pesticide in the cigarette is not uniformly spread over all of the cigarette. This adds to the importance of our study since our cigarettes had tobaccos in which the MH concentration was uniform throughout.

EXPERIMENTAL PROCEDURES

Materials. All cigarettes, whether purchased or made from tobacco specially grown for the study, were stored at 0 °C until required for use.

The 1R1 cigarettes were purchased in 1970, and the commercial cigarettes, all different brands, were purchased in 1968 and 1980 from local grocery stores on the day their shipment arrived at the stores.

Description of Tobaccos Specially Grown. (1) Tobacco, variety Coker 347, was planted on May 10, 1973, in a field at Chinqua Penn, NC. Royal MH-30 (active ingredient K-MH) was applied to the tobacco. A detailed description

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Table I. Cigarettes, Their Lengths and Weights, Weights of Cigarette Butts and Ashes, and Maleic Hydrazide (MH) Residues in Cigarette and Cigarette Butt Tobacco and Main-Stream and Side-Stream Smokes

						MH residues in				
		cig. length,	weight, g, of			cig. tob.,	butt tob.,	ashes,	main stream,	side stream,
no.	form of MH in cig. tob.	mm	cig.	butts	ashes	μg/g	μg/g	μg/g	μg/40 cig.	μg/40 cig.
1	Royal MH-30 (K-MH), treatment A	70	0.810	0.313	0.0712	176.0	135.0	ND[a]	48.0	37.1
2	Royal MH-30 (K-MH), treatment B	70	0.799	0.301	0.0624	161.0	132.0	ND	78.4	10.4
3	MH (acid form), treatment A	70	0.825	0.312	0.0651	154.0	125.0	ND	80.8	8.0
4	MH (acid form), treatment B	70	0.817	0.304	0.0612	13.0	11.8	ND	6.9	ND
5	MH-30 (DEA-MH)	85	1.180	0.355	0.1507	35.0	46.3	ND	66.9	40.2
6	1R1	85	1.048	0.352	0.1263	102.0	62.3	ND	65.2	56.0
7	1968 purchase A	85	1.011	0.355	0.1416	34.4	44.0	ND	38.0	31.8
8	1968 purchase B	85	0.988	0.353	0.1507	9.2	14.7	ND	8.0	6.0
9	1980 purchase A	85	1.046	0.355	0.1296	146.0	85.3	ND	76.3	49.0
10	1980 purchase B	70	0.81	0.319	0.0687	178.3	135.7	ND	83.3	38.0

[a] Not detected.

of various treatments, etc., are given in our previous publication (Chopra and Mahfouz, 1977). Cigarettes no. 1 (see Table I) were made from this tobacco. (2) Tobacco, variety Coco 319, was planted on May 12, 1971, in a field at Oxford, NC in random squares. At different dates the squares were treated with Guthion (1 pt/acre), Thiodan (1–1.5 pt/acre), and Sevin [80% (w/w), 1–1.25 lb/acre]. To some of these squares Royal MH-30 (K-MH, 2.25 lb/acre) was applied; tobacco plants on other squares were hand suckered. Cigarettes no. 2 were made from Royal MH-30 treated tobacco and "check" cigarettes, (i.e., cigarettes containing no MH). Cigarettes no. 3 were made from hand-suckered tobacco to which appropriate quantity of MH (acid form) was applied at the time of its manufacture. Cigarettes no. 4 were made in the same way. (3) Tobacco, variety N.C. 2326, was planted in April 1970 at Border Belt Tobacco Research Station, Whiteville, NC. Prior to planting the field was fumigated with Terricide 15D (6 gal/acre), and later on, the plants were treated with six applications of Sevin [1 lb/acre, 80% (w/w)] and one application each of Malathion (1 qt/acre), Off-Shoot T (2 gal/acre), and MH-30 (diethanolamine salt, 1 gal/acre). Cigarettes no. 6 were made from these tobaccos. All the tobacco leaves were flue cured in the conventional method before they were manufactured into cigarettes.

Smoking Machine. The diagram of the smoking machine is shown in Figure 1. The relevant parts of the smoking machine consist of a turn plate 1.2 cm thick, having 30 holes equidistant from each other. Each of these holes could snugly hold a cigarette. The turn plate could make a complete revolution in 1 min, with each revolution consisting of 30 stops of 2-s duration. At each stop, 1 of the 30 holes stops in front of a funnel through which the cigarette smoke (main stream) produced could be channeled to pass through a cambridge filter pad and a train of traps.

The turn plate itself is enclosed in a stainless steel chamber with an opening in front of the funnel to permit air in. The chamber can open circumferentially into two parts to permit cleaning of the chamber, reloading of the cigarettes on the turn plate, etc. The side-stream smoke produced during the smoking is channeled through an exhaust pipe, a cambridge filter pad, and a train of traps.

Methods. *Sampling.* A pool of 600 cigarettes each was created for all the different types of cigarettes, and aliquots from these pools were taken for MH analyses in cigarette tobacco, main-stream and side-stream smokes, cigarette butt tobacco, and ashes as described below.

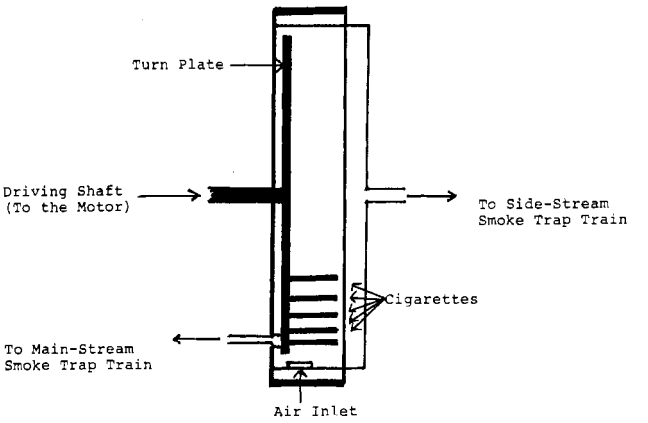

Figure 1. Plan of the smoking chamber of the smoking machine.

For MH analyses in cigarette tobaccos, the wrapping paper from 10 cigarettes from the pool was removed and the tobacco of the cigarettes mixed. Aliquots of 1 g each from the tobacco mixture was used for MH analyses in the tobacco. For MH analyses in main-stream and side-stream smokes, 40 cigarettes per analyses were taken from the pool and kept in a humidifying chamber for 24 h at 66% humidity. These cigarettes were then smoked in eight lots of five cigarettes each as described below, and their main-stream and side-stream smoke condensates were collected for MH residue analyses. Ashes and butts tobaccos from these smoked 40 cigarettes were also collected and pooled together (separately) and weighed (see Table I). Aliquots of 1 g each were taken from the pooled ash and butts tobacco sample and analyzed for MH residues the same way as the tobaccos from cigarette were analyzed.

Smoking of Cigarettes. Cigarettes were smoked on the cigarette smoking maching under F.T.C. conditions: i.e., puff volume 35 mL, puff duration 2 s, puff frequency one/min, butt length 23 mm, and tobacco moisture contents 12%. The machine was standarized against a P. Lorillord Tobacco Co., Greensboro, NC, 20-port automated cigarette smoking maching designed by Phillip Morris Tobacco Co. and manufactured by Phipps and Bird, Richmond, VA.

In all 40 cigarettes were smoked for each analysis. The main-stream and side-stream smokes were collected at the same time. These smokes were collected in two separate trains, each consisting of a cambridge filter followed by

three traps containing about 60 mL, all told, of a 2 N NaOH solution at 0 °C. At one time only five cigarettes were smoked, the cambridge filter were then changed (the traps and their contents were not changed), and a fresh lot of five more cigarettes were smoked. This process was repeated until all 40 cigarettes were smoked; all the surface area of the smoking machine which was exposed to the side-stream smokes was wiped with a cambridge filter pad soaked with 2 N NaOH so that any MH on these exposed surface areas could be collected. This cambridge filter pad was then added to the other eight cambridge filter pads from the side-stream smokes, and the eight from the main-stream smokes were then extracted separately with 20 mL of 2 N NaOH and the extracts were then mixed with appropriate solutions in the traps. The combined mixture for main-stream and side-stream smokes were then concentrated separately to about 50 mL by heating them on a vacuum rotary evaporator at diminished pressure, and their MH contents were estimated according to the method described below.

Analysis of MH in Cigarette and Cigarette Butt Tobacco. MH in cigarette and cigarette butt tobaccos was estimated according to the method of Lane (1963) as modified by Nesemann et al. (1974).

This method was also used for MH determination of ashes and main-stream and side-stream smokes. For ashes, a sample of 1 g of ashes was taken, and for the main-stream and side-stream smokes, the whole of their respective concentrates from the NaOH solutions in the traps plus NaOH extracts of the respective cambridge filter pads was used for the analysis as mentioned above.

Recovery curves of MH in cigarette tobacco, ashes, and main-stream and side-stream smokes were made. They were all linear over the range of experimentation. MH residue values reported in all instances were fairly reliable at the 5-ppm level, becoming even more reliable as MH residue values increase from that figure. Residues values below 5 ppm were in all instances only approximate. All MH residue values were determined in 1980–1981.

Preparation of DEA-MH. MH (1 g = 0.0089 mol) was suspended in 25 mL of water, and 25 mL of solution containing 1 g (0.0095 mol) of diethanolamine was added to it when a clear solution was formed. Water was removed from the reaction mixture by heating it under diminished pressure. This resulted in a clear, colorless, viscous, sticky substance which defied all attempts to crystallize it.

Relative Volatility of Acid-MH, DEA-MH, and *K-MH.* Aliquots of approximately 1 mg of acid-MH (i.e., 99+% pure $C_4H_4N_2O_2$), DEA-MH (sticky viscous material obtained from the above-mentioned experiment), and K-MH (supplied by Uniroyal Chemical Co.) were applied at three different location on a circular glass slide (1-cm diameter) and heated on a Fisher-Johns melting point apparatus. Acid-MH started subliming at about 280 °C and completely sublimed off by 300 °C. There was no blackening of the substance [MH-acid form, melts at 307–307.5 °C (Smith et al., 1977)]. At 220 °C, a few bubbles appeared in DEA-MH. At 230 °C it started turning brown and became an almost black sticky liquid by 240 °C. Some disappearance of the material was also seen. K-MH did not show any change even when heated beyond 300 °C.

RESULTS

The results of our estimations are given in Tables I–III. Table I gives the length of the cigarettes, the weight of the cigarettes, butts, and ashes, and MH residues in cigarette tobacco, butts, and ashes (micrograms per gram) and in main-stream and side-stream smokes (micrograms per 40 cigarettes).

Table II. Fate of Maleic Hydrazide (MH) in Smoking Cigarette and the MH Contents of Cigarette, Cigarette Butts, Tobacco Ashes, and Main-Stream and Side-Stream Smokes

no.	form of MH in cig. tob.	MH residues in cigarette, μg: tob. (P)	butt portion (Q)	ash portion (R)	MH in main-stream smoke, μg/cig. (S)	MH in side-stream smoke, μg/cig. (T)	theoretical amt of MH in cig. butts μg/butts (U)	MH tob. smoked, μg (V)[a]	MH not destroyed during smoking, μg (W)[b]	% MH survival ($W/V \times 100$)
1	Royal MH-30 (K-MH), treatment A	142.6	42.4	ND[c]	1.2	1.0	46.9	95.7	1.2	1.23
2	Royal MH-30 (K-MH), treatment B	128.8	39.8	ND	1.5	0.3	42.9	85.9	1.7	2.00
3	MH (acid form), treatment A	123.2	39.1	ND	2.0	0.2	41.1	82.1	2.2	2.80
4	MH (acid form), treatment B	10.6	3.4	ND	0.2	ND	3.5	7.0	0.2	2.48
5	MH-30 (DEA-MH)	41.3	16.4	ND	1.7	1.0	11.0	30.2	8.1	26.90
6	1R1	107.1	21.9	ND	1.6	1.4	28.9	78.2	3.0	3.88
7	1968 purchase A	34.7	15.4	ND	1.0	0.8	9.3	25.4	9.0	35.49
8	1968 purchase B	9.2	5.1	ND	0.2	0.0	2.5	6.7	2.9	43.06
9	1980 purchase A	154.7	30.0	ND	1.9	1.2	41.8	112.9	3.5	2.30
10	1980 purchase B	144.4	43.2	ND	2.1	1.0	48.0	96.4	3.8	4.00

[a] $V = P - U$. [b] $W = S + T + (Q - U)$. [c] Not detected.

Table III. Maleic Hydrazide Transfer Rates in Cigarette Main-Stream and Side-Stream Smokes

no.	form of MH in cig. tob.	cig. length, mm	MH concn, in tobacco, μg/g	transfer rate/g of tob. smoked, % main stream	transfer rate/g of tob. smoked, % side stream
1	Royal MH-30 (K-MH), treatment A	70	176.0	1.25	0.97
2	Royal MH-30 (K-MH), treatment B	70	161.0	1.69	0.30
3	MH (acid form), treatment A	70	154.0	2.46	0.24
4	MH (acid form), treatment B	70	13.0	2.83	ND[a]
5	MH-30 (DEA-MH)	85	35.0	5.54	3.33
6	1R1	85	102.0	2.09	1.80
7	1968 purchase A	85	34.4	3.80	3.20
8	1968 purchase B	85	9.2	2.99	2.45
9	1980 purchase A	85	146.0	1.69	1.15
10	1980 purchase B	70	178.0	2.16	0.99

[a] Not detected.

Table II gives the MH residues (micrograms per cigarette) in cigarette tobacco, butts, ashes, and main-stream and side-stream smokes. It also gives the theoretical amount of MH in butts calculated as

MH in cigarette butts = 23 mm × (amount of MH in whole cigarettes)/(length of the cigarette in mm)

The amount of MH present in the portion of cigarette smoked, i.e., not destroyed during smoking, equals MH residues in main-stream smoke plus MH residues in side-stream smoke plus excess of MH residues in butts over the theoretical amount of MH present [i.e., $W = S + T + (Q - U)$].

Table III gives cigarette lengths, amount of MH (micrograms per gram) present in cigarette tobacco, and the MH main-stream and side-stream transfer rates (calculated on the basis of the amount of MH residues given off by tobacco actually smoked).

DISCUSSION

The transfer rates for MH in the main-stream and side-stream smokes (Table III) largely depend upon the form of MH used, their range being 2.46–2.83% for main-stream and 0.29%–not detected for side-stream smokes for cigarettes containing MH in the acid form, 1.25–1.69% for main-stream and 0.3–0.97% for side-stream smokes for cigarettes containing MH in the K-MH form, and 5.54% for main-stream and 3.33% for side-stream smokes for cigarettes containing MH in DEA-MH form. Since K-MH is less volatile than DEA-MH and Acid-MH, a pattern between the volatility of the MH form and the transfer rates of MH in main-stream smokes may be seen. This result is consistent with the results of Atallah and Dorough (1975) and our earlier findings (Chopra and Thekkekandam, 1973). A study on the relative volatility of DEA-MH and acid-MH was inconclusive. However, we assume that under smoking conditions DEA-MH is more volatile than acid-MH.

The pattern of MH residue concentration in butts is the most interesting. In cigarettes containing K-MH and acid-MH the concentration of MH in the butt tobacco after smoking is less than it was before smoking (Table I). This, apparently, is due to the retention of moisture and particulate matter, etc., by the butt segment during the process of smoking. Haeberer and Chortyk (1974) report similar results. However, in the instance where DEA-MH was used, there is a considerable increase in the concentration of MH in the butts. This pattern would also be expected.

Another interesting, and expected, finding is the amount of MH present in ashes. The temperatures in the burning zone of the cigarette reach up to 900 °C [cf. Touey and Mumpower (1957)], and it would be surprising if MH could survive temperatures that high.

Our main-stream MH transfer rates are also consistent with the few published results. The commercial cigarettes containing 31.2 ppm of MH (length 85 mm) purchased and smoked by Liu and Hoffmann (1973) gave main-stream MH transfer rates of 3.94%. This is in an excellent agreement with the main-stream MH transfer rate of 3.80% we got with our cigarette purchased in 1968, which had a length of 85 mm and MH content of 34.4 ppm. However, Liu and Hoffmann (1973) also report a transfer rate of 7% and 10.3%; our highest rate is 5.54% for a cigarette containing DEA-MH. We have no explanation as to why Liu and Hoffmann had such high transfer rates. Our main-stream MH transfer rates of 1.16% are also consistent with those of Davis et al. (1977, 1979).

We do not have any comparative study on the cigarette length and the main-stream transfer rate, though from the only one example (Table III), that is, of 1980 commercial cigarettes, it appears that the transfer rates for the shorter cigarettes are higher than those for the longer cigarettes.

We also could not find any information on side-stream MH transfer rates. However, once again our results show that the more volatile DEA-MH has a higher transfer rate.

Comparing the 1968 cigarettes with 1980 cigarettes, we observe two trends: (1) the falling of main-stream MH transfer rates to even more than 57%, from 3.94% to 1.69% and (2) an increase of MH residues in cigarette tobacco. From our studies, the former trend would suggest that there has been a shift from DEA-MH to K-MH during this period. This is indeed what has happened [cf. Steffens (1979)].

In conclusion, a change from more volatile DEA-MH to less volatile K-MH has brought a reduction in the main-stream MH transfer rate. Since the most important consideration is not how much pesticide is in tobacco but how much pesticide is in main-stream smokes, any tolerances on MH, which were based on results obtained 5 or 8 years ago, are now out of date and should be reexamined. A cigarette containing 80 ppm of MH and a transfer rate of 5% would give the same amount of MH in the main-stream smoke as a cigarette containing 240 ppm of MH with a transfer rate of 1.6% would. Further, a reduction in the amount of pesticide in the cigarette main-stream smokes can be accomplished by a judicious use of the form

and rate of the pesticide applied.

It might be of interest to mention a few words about the degradation products of MH in tobacco smokes. Works of Liu and Hoffmann have shown that MH does not contribute to any significant extent to the hydrazine present in tobacco smokes. Patterson et al. (1978) found benzo[a]pyrene in the pyrolyzate of MH when neat MH was prolyzed. This led to the possible implication that MH in cigarette tobacco could also contribute to benzo[a]pyrene in tobacco smokes. We, however, on mathematical grounds, have questioned this implication (Chopra, 1979). Ninety-four percent of MH is known to degrade into CO_2, CO, NH_3, HCN, etc. (Smith et al., 1977), and no one, so far, to our knowledge has experimentally implicated MH with PAH's.

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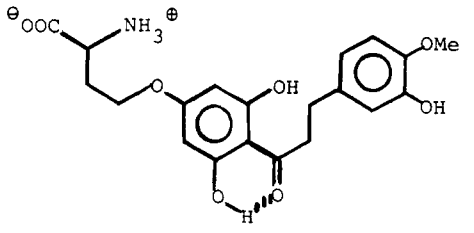
Dihydrochalcone Sweeteners. Synthesis, Sensory Evaluation, and Chiral Eluant Chromatography of the D and L Antipodes of a Potently Sweet, Sucrose-like Homoserine–Dihydrochalcone Conjugate

Grant E. DuBois*[1] and Rebecca A. Stephenson

The D and L antipodes of the potently sweet homoserine–dihydrochalcone conjugate 2,3′,6-trihydroxy-4-(3-amino-3-carboxypropoxy)-4′-methoxydihydrochalcone (1) were synthesized by alkylation of hesperetin (2) with D and L iodides **15**, followed by hydrogenation in alkali. The D and L alkylating agents were prepared in six steps from D- and L-methionine, respectively. Enantiomeric purity was determined unambiguously by chiral eluant high-performance liquid chromatography employing a Cu(II)–L-Asp-L-Phe-OMe system. L-1 was determined to have a solubility 1.8 times that of racemic 1. Enantiomerically pure D-1 and L-1 were determined by sensory panel studies to have taste potencies and qualities comparable to those of DL-1, showing the side chain chirality of 1 to have no effect on sensory properties.

Recently we reported the synthesis and sensory evaluation of 2,3′,6-trihydroxy-4-(3-amino-3-carboxypropoxy)-4′-methoxydihydrochalcone (1), the first analogue of the well-known potently sweet flavanoid glycoside neohesperidin dihydrochalcone which has rigorously been demonstrated to have significantly diminished aftertaste (DuBois et al., 1981a). Unfortunately, however, a rather



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low solubility (45.3 mg/L at 23 °C) places a limit on the utility of 1 in many food systems. It is generally true that

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