Flavonoid Sweeteners. Synthesis and Intestinal Absorption of Selected Sulfoalkylated Hesperetin-3-^{14}C Derivatives in the Rat

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The intestinal absorption of four sulfoalkyl derivatives of hesperetin-3-^{14}C and hesperetin dihydrochalcone-β-^{14}C (DHC) was determined with bile duct ligated rats. While absorption, as indicated by urinary excretion, $^{14}CO_2$ expiration, and residual tissue activity, is nearly complete for hesperetin-3-^{14}C (91% of dose), the sulfoalkyl derivatives exhibited apparent minimal metabolism and a greatly reduced intestinal absorption. The absorption was found, as expected, to be inversely proportional to molecular weight and charge. Absorption ranged from 23% for 7-O-sulfopropylhesperetin-3-^{14}C (mol wt 424, acid form) to 1.2% for a DHC dimer (mol wt 985, acid form). These results prove feasible the concept of preparing relatively low molecular weight, nonglycosidic flavonoid sweeteners possessing limited intestinal absorption and metabolism.

There is little disaggreement among researchers that a genuine need for a biologically safe sucrose substitute exists (Crosby et al., 1978b). This need is derived from both the increased recognition of sucrose-related health problems (Yudkin, 1972; Shaw and Roussos, 1978) and the continued uncertainty over the safety of saccharin and many of its alternatives (Berg, 1975; Kramers, 1975; Howe et al., 1977; Crosby and Wingard, 1978). Our "anatomical compartmentalization" approach to nonnutritive sweeteners employs nonglycosidic dihydrochalcones (DHCs) in conjunction with molecular modifications intended to restrict or virtually eliminate intestinal absorption (Crosby et al., 1978a). Limited intestinal absorption should reduce the possibility of chronic toxic effects developing through normal use.

We report here rat intestinal absorption studies conducted with ^{14}C-labeled analogues of 7-O-sulfopropylhesperetin (Crosby et al., 1978c), 4-O-sulfopropylhesperetin DHC (DuBois et al., 1977b), and a *core concept* DHC dimer (Crosby et al., 1978a); all of which have been shown to be sweet in the unlabeled form. Also included in this study, as a model low-molecular-weight DHC disulfonate, was a labeled analogue of tasteless 2,4-di-O-sulfopropylhesperetin DHC (Dubois et al., 1977b). These four compounds provided illuminating data concerning the effects of alkyl substitution and charge on both the intestinal absorption and metabolism of 400–1000 mol wt nonglycosidic flavonoid sweeteners.

SYNTHETIC PROCEDURES

General. Liquid scintillation counting was performed with a Packard Tri-Carb Model 2420 instrument. Analytical thin-layer chromatography (TLC) was carried out on prelayered silica gel 60 F-254 plates (E. Merck A. G., Darmstadt, Germany) of layer thickness of 0.25 mm. The plates (5 × 20 cm) were developed to 12 cm and examined with a Packard Model 7201 radioscanner. All reactions, except hydrogenation, were conducted under an argon atmosphere and the chemicals employed were anhydrous reagent grade used as received unless otherwise indicated. Dimethylformamide (DMF) was distilled from CaH_2 (20 mm) and stored over molecular sieves under Ar. Vacuum transfer and drying operations were performed at room temperature and 0.1 μ.

The proton nuclear magnetic resonance (^1H NMR) spectrum was recorded with a Varian T-60A spectrometer and is reported as parts per million (δ) relative to internal tetramethylsilane. The combustion analysis was carried out by the Microanalysis Laboratory, Stanford University, Stanford, Calif.

Gradient-elution, high-pressure liquid chromatography (LC) was performed with a Waters Associates (Milford, Mass.) instrument constructed from a U6K universal injector, a Model 660 solvent flow programmer, and two Model 6000 pumps. The detector was a Model SF 770 spectroflow monitor (Schoeffel Instrument Corp., Westwood, N.J.) equipped with a Model GM 770 monochromater operating at 286 nm. Analytical work was conducted with a Waters Associates 30 cm × 4 mm i.d. reverse-phase μ Bondapak C-18 column eluted with a linear (program 6) gradient (15.0 min, 2.0 mL/min) of 10 to 100% spectrophotometric grade methanol in 0.03 M KH_2PO_4 buffer (Seitz and Wingard, 1978). Preparative work was done with a Waters Associates Porasil B C-18 column ($^3/_8$ in. × 4 ft) eluted with a linear gradient (60 min, 8 mL/min) of 10 to 100% methanol in distilled H_2O.

1,3-Propane sultone (**2**) has been shown to be a potent carcinogen in animals (Doak et al., 1976). Reaction mixtures containing **2** and disultone **7** should be handled with care.

3′,5-Dihydroxy-4′-methoxy-7-(3-sulfopropoxy)-flavanone-3-^{14}C, Potassium Salt (3). Hesperetin-3-^{14}C (**1**, 15.4 mg, 0.051 mmol, 99.8 μCi), contained in 0.5 mL of DMF, was treated with 12.0 mg (0.098 mmol) of distilled [bp 95–96 °C (0.05 mm)] 1,3-propane sultone (Aldrich Chemical Co., Milwaukee, Wis.) and 7.4 mg (0.05 mmol) of K_2CO_3 for 24 h at ambient temperature. The DMF was removed by vacuum transfer and the residue dissolved in H_2O (2 mL), washed with ethyl acetate (3 × 2 mL), and passed through a 0.8-μm filter. Preparative LC provided a homogeneous fraction (6.47 μCi), chromatographically identical with an unlabeled sample of **3** (DuBois et al., 1977b), for use in the absorption study.

2,3′,6-Trihydroxy-4-(3-sulfopropoxy)-4′-methoxydihydrochalcone-β-^{14}C, Potassium Salt (4). Hesperetin-3-^{14}C (53.2 mg, 0.176 mmol, 345 μCi) was dissolved in 1.0 mL of DMF and treated with 39.4 mg (0.323 mmol) of distilled **2** and 24.3 mg (0.176 mmol) of K_2CO_3 as before. TLC indicated quantitative conversion to **3** (R_f 0.60, ethanol). After workup, the aqueous solution (2 mL) was treated with 100 mg of KOH and 25 mg of 5% Pd on C and reduced under 24 psi H_2 (5 h, 25 °C). The solution was acidified to pH 5 with 6 N HCl and the catalyst removed by filtration. Partial evaporation (Ar stream), followed by crystallization, centrifugation, and drying,

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afforded 11.9 mg (46.5 μCi) of white crystalline 4 (>98% pure by LC) for absorption study use.

2,4-Bis(3-sulfopropoxy)-3′,6-dihydroxy-4′-methoxydihydrochalcone-β-^{14}C, Dipotassium Salt (5). The mother liquor from the crystallization of 4 (above) was evaporated to give 198 mg of off-white solid containing inorganic salts. Trituration (DMF, 1 mL) and filtration provided a filtrate shown by TLC to contain radiochemically pure 4 (R_f 0.50, ethanol). A portion of the solution (0.535 mL, 92.5 μCi) containing 0.051 mmol of 4 was treated portionwise with 20.7 mg (0.170 mmol) of distilled 1,3-propane sultone and 21.3 mg (0.153 mmol) of K_2CO_3 over a period of 12 days. TLC indicated 80% conversion to 5 (R_f 0.42, isobutyl alcohol–acetic acid–water, 2:1:1). The fine, tan precipitate was isolated by centrifugation, rinsed with DMF (2 × 1 mL, 0 °C), and dried to provide 42.6 mg of solid. Recrystallization from glacial acetic acid afforded, after washing with methanol (2 × 0.5 mL) and drying, 6.3 mg of 5 as a tan solid found to be >97% pure by LC and of equivalent radiochemical purity by TLC. Radioassay gave a specific activity of 3.13 μCi/mg (1.96 mCi/mmol; cf. hesperetin-3-^{14}C sp act. 6.48 μCi/mg, 1.96 mCi/mmol).

Hesperetin Dihydrochalcone-β-^{14}C Dimer 8. A 1-L, one-neck flask, equipped with magnetic stir bar and septum cap, was charged with 9.15 g (75.0 mmol) of distilled 2 and 375 mL of tetrahydrofuran (distilled from lithium aluminum hydride), flushed with Ar, and cooled to −78 °C. At this temperature, the solution was treated with 82.5 mmol of *n*-butyllithium in hexane over 15 min and, after stirring 5 min, with a solution of 3.35 g (25.0 mmol) of terephthalaldehyde (recrystallized from toluene) in 25 mL of tetrahydrofuran. After stirring 1 h at −78 °C, the mixture was poured into 300 mL of saturated aqueous NaCl containing 84 mmol of HCl, and the layers were separated. The organic layer was dried ($MgSO_4$), evaporated to 50 mL, cooled at 0 °C overnight, and filtered to provide 854 mg (9.0%) of disultone 7 as a white crystalline solid: uncorrected mp 188.5–189.0 °C (dec); ^1H NMR (Me_2SO-d_6) 2.23–2.80 (m, 4, CCH_2C), 3.43–3.97 (m, 2, SO_2CH), 4.07–4.70 (m, 4, OCH_2), 4.92 (d, J = 6 Hz, 2, benzal CH), 5.68 (br s, 2, OH), and 7.42 (s, 4, aromatic). Anal. Calcd for $C_{14}H_{18}O_8S_2$: C, 44.44; H, 4.79; S, 16.95. Found: C, 44.62; H, 4.94; S, 16.68.

A solution of 1 (36.2 mg, 0.119 mmol, 235 μCi) in 0.75 mL of DMF was treated with 19.6 mg (0.052 mmol) of 7 and 17.4 mg (0.126 mmol) of K_2CO_3 for 9 days at room temperature. The DMF was removed by vacuum transfer and the gummy residue dissolved in 2 mL of H_2O, treated with 0.25 mmol of HCl, and extracted with ethyl acetate (10 × 2 mL). Potassium hydroxide (94 mg) and 5% Pd on C (25 mg) were added, and the solution was hydrogenated at 38 psi H_2 (20 h, 25 °C). After removal of the catalyst and acidification, lyophilization gave 148 mg of tan solid which was subjected to preparative LC. A fraction containing 20.5 μCi of DHC dimer 8 of >99% purity by LC and TLC (R_f 0.50, isobutyl alcohol–acetic acid–water, 2:1:1) was obtained for the absorption studies.

MATERIALS AND METHODS

Animal Experiments. Doses of 1.2 to 1.7 μmol of the test agents were prepared by dissolving in distilled water. Each 1 mL dose contained 2–3 μCi of radiolabeled compound. Female Simonsen/Sprague-Dawley rats (Simonsen Laboratories, Gilroy, Calif.) weighing 170–190 g were used. Each rat received a 1-mL dose of the [^{14}C]hesperetin derivative by gavage (Table I). Animals designated for CO_2 collection were immediately placed in a special collection chamber. Most of the animals were bile duct ligated by the method of Cameron and Oakley (1932). All animals had free access to food (Simonsen white diet) and water with the exception of an overnight fast prior to dosing. Food was returned 8 h after dosing.

Table I. Rat Preparation and Dosage

study	surgical preparation	no. of animals	dosage route	dose administered mg	dose administered μCi
I	bile duct ligated	2	oral	0.55	2.14
IIa	none	4	oral	0.72	2.82
IIb	bile duct ligated	4	oral	0.72	2.82
III	bile duct ligated	5	oral	0.68	2.12
IV	bile duct ligated	5	oral	1.17	2.15

Urine and fecal samples were separated and collected at 24-h intervals from intact and bile duct ligated animals housed in stainless steel metabolism cages. The expired CO_2 from animals housed in stainless steel metabolism cages enclosed in air-tight plexiglass chambers was collected by pulling room air through the chambers and through two sequentially linked gas washing bottles containing Carbosorb II (Packard Instrument Co., Downers Grove, Ill.). The solutions were changed at 24-h intervals.

All rats were sacrificed 96 h after dose administration. The liver, spleen, kidneys, stomach, small intestine, cecum, large intestine, and a small periuterine fat sample were taken for radioassay after sacrifice. Organ and tissue samples were stored at −20 °C until radioassay.

Radioassay of Biological Samples. Urine collections were weighed and for intact animals (study IIa) 0.5 mL was placed directly into 10 mL of Instagel (Packard Instrument Co.). The urine samples (0.25 mL) of the bile duct ligated animals were treated with an equal volume of 30% aqueous H_2O_2 and, after 1 h, diluted with 10 mL of Instagel. Fecal samples were collected in preweighed plastic bags and soaked overnight at 4 °C in three weights of H_2O. After homogenization, aliquots (0.2–1.0 g) were weighed into Combustocones (Packard Instrument Co.) and dried at 60 °C. The contents were rinsed from the gastrointestinal tract with physiological saline and homogenized, and aliquots were weighed into Combustocones and dried. Tissues, with the exception of liver samples, which were first pulped, were weighed directly into Combustocones and dried.

Samples of tissue, organs, gut contents, and feces were oxidized in a Packard Tri-Carb Model 306 sample oxidizer, and the liberated CO_2 was trapped in 10 mL of Oxisorb-2 (New England Nuclear, Boston, Mass.). The solutions were diluted with 10 mL of Oxiprep-2 (New England Nuclear) in liquid scintillation vials. Cage washings were weighed and 1.0-mL aliquots were added to 10 mL of Instagel.

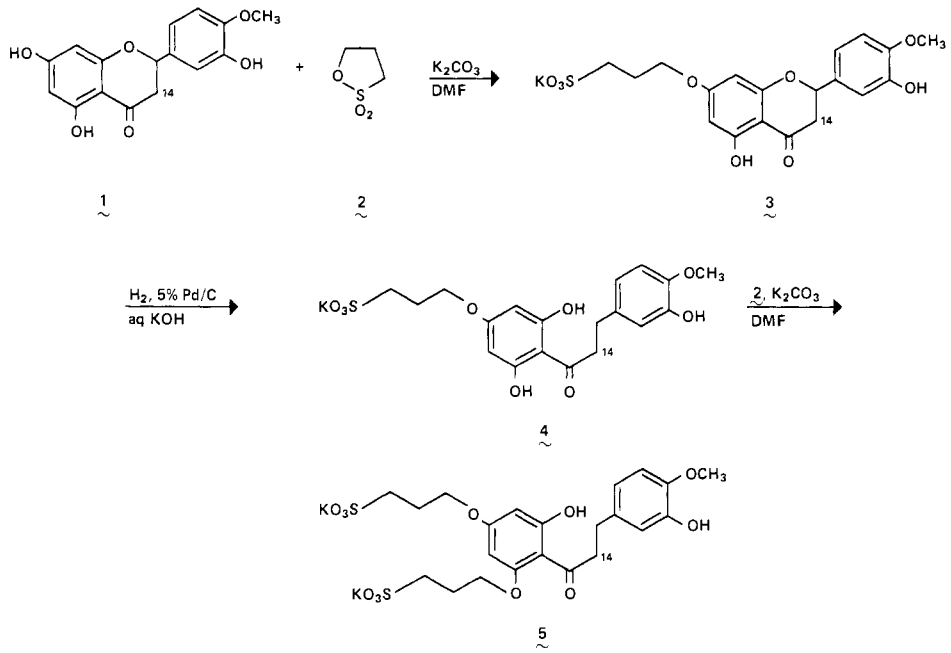
A Packard Tri-Carb Model 3385 liquid scintillation spectrometer was employed for the radioassay of biological samples. Absolute radioactivity was estimated with efficiency correlation curves based on an automatic external standard.

Detection and Characterization of Urine Metabolites and Cecal Microflora Incubation. These experiments were carried out with the 4-*O*-sulfopropylhesperetin dihydrochalcone-β-^{14}C according to the methods described by Honohan et al. (1976).

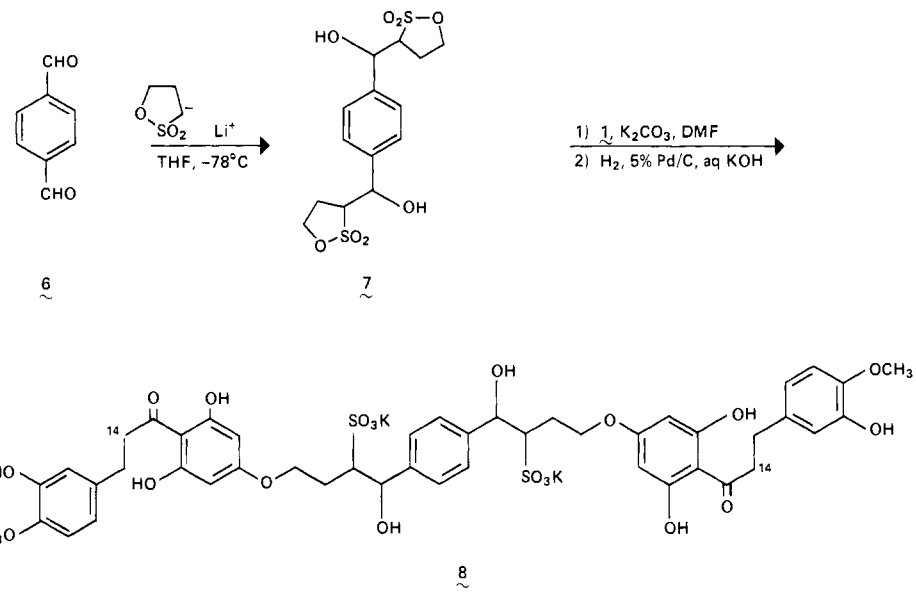
RESULTS

Synthetic Considerations. The sequential synthesis of radiolabeled flavonoids 3-5, which follows our previously reported preparations of the unlabeled analogues (DuBois et al., 1977b), is illustrated in Scheme I. The sequence began with the preparation of 7-*O*-sulfopropylhespere-

Scheme I



Scheme II



tin-3-^{14}C (3) via the regioselective alkylation of hesperetin-3-^{14}C (Honohan et al., 1976) with 1,3-propane sultone (2). The alkaline hydrogenation of 3 then provided a labeled version (4) of the intensely sweet 4-*O*-sulfopropylhesperetin DHC. In the final step, the difference between the acidities of the three phenolic hydroxyls of 4 was used to advantage for the preparation of disulfopropyl derivative 5. The factors affecting alkylations such as those in Scheme I have recently been discussed (DuBois et al., 1977a).

Scheme II depicts the preparation of a radiolabeled DHC dimer (Crosby et al., 1978a). Durst and du Manoir reported the preparation of a variety of substituted sultones from 2 by generation of the α-lithio carbanion (*n*-butyllithium, −78 °C) and then treatment with electrophiles (1969). The use of terephthalaldehyde (6) as electrophile provided disultone 7 in modest yield. The coupling of 2 equiv of hesperetin-3-^{14}C to the disultone, followed by alkaline hydrogenation and preparative LC, afforded labeled DHC dimer 8 in a high state of purity.

Excretion of Radioactivity and Intestinal Absorption. For the test compounds, excretion of radioactivity from all routes (urine, feces, and expired CO_2) was maximum during the 24 h following dosing and was essentially complete within 48 h. Recoveries of radioactivity for the test compounds, expressed as a percentage of the administered dose, are presented in Table II. Also given in Table II is the comparable data for hesperetin-3-^{14}C. The latter compound is largely excreted via the urine and expired CO_2, indicating that it is nearly completely absorbed and metabolized. However, intestinal absorption of the hesperetin-3-^{14}C derivatives, as indicated by the radioactivity found in urine, internal organs, and expired CO_2 of bile duct ligated rats, was found to decrease sharply with sulfopropylation of the A ring and reductive opening of the dihydropyrone ring. Thus sulfopropylated hesperetin was 23.2% absorbed, sulfopropylated hesperetin DHC <12.2% absorbed, and disulfopropylated hesperetin DHC <4.7% absorbed. A "dimer" of the sulfopropylated hesperetin DHC with a formula weight of 985 (free di-

Table II. Recovery of Radioactivity after Oral Administration of Hesperetin-3-^{14}C and Its Derivatives to Rats

test agent	study	% of administered dose[a] urine	feces	internal organs	gut contents	expired CO_2	cage washings	total recov.[b]
hesperetin-3-^{14}C (1) (MW 302)		33.2 ± 6.5[f]	14.8 ± 6.3	1.4 ± 0.5	<0.1	39.2[c]	0.9 ± 0.1	50.6 ± 11.0
		83.8 ± 5.6[e,f]	4.1 ± 1.1	0.4 ± 0.1	<0.1	6.3[c]	1.0 ± 0.2	89.3 ± 5.6
7-O-sulfopropyl-hesperetin-3-^{14}C (3) (MW 424)	I	22.8 ± 8.8[e]	57.3 ± 23.1	0.1 ± 0.1	9.3 ± 13.1	0.3 ± 0.2[d]	0.2 ± 0.1	90.2 ± 18.6
4-O-sulfopropyl-hesperetin dihydrochalcone-β-^{14}C (4) (MW 426)	IIa	0.5 ± 0.04	95.8 ± 1.6	<0.1	<0.1	0.1[c]	0.2	96.3 ± 1.6
	IIb	12.0 ± 3.6[e]	82.7 ± 2.4	<0.1	<0.1	0.2[c]	1.1 ± 1.3	95.6 ± 1.8
2,4-di-O-sulfopropylhesperetin dihydrochalcone-β-^{14}C (5) (MW 548)	III	4.4 ± 0.4[e]	92.6 ± 2.3	<0.1	0.1 ± 0.1	<0.1[d]	0.4 ± 0.2	97.5 ± 2.3
4-O-sulfoalkyl-hesperetin dihydrochalcone-β-^{14}C "dimer" 8 (MW 985)	IV	1.00 ± 0.2[e]	96.9 ± 4.8	<0.1	0.4 ± 0.4	<0.1[d]	0.2 ± 0.2	98.5 ± 4.6

[a] Data are group means ± standard deviation. [b] Expired CO_2 is not included. [c] Data are from single rats. [d] Data are from two rats. [e] Bile duct ligated. [f] Data from Honohan et al., 1976.

Table III. Incubation of 4-O-Sulfopropylhesperetin Dihydrochalcone-β-^{14}C with Rat Cecal Microflora

sample	concn, mg/mL	sp act, μCi/mg	metabolic products[a,b] R_f 0.05	R_f 0.20	R_f 0.47	R_f 0.60	R_f 0.70
4-O-sulfopropyl-hesperetin DHC-β-^{14}C	5.0	0.018	99	ND[c]	ND	1.0	ND
control	5.0	0.018	98	ND	ND	1.6	ND
4-O-sulfopropyl-hesperetin DHC-β-^{14}C	0.5	0.18	100	ND	ND	ND	ND
control	0.5	0.18	92.5	ND	ND	7.5	ND
4-O-sulfopropyl-hesperetin DHC-β-^{14}C	0.015	6.03	97	ND	ND	ND	2.8[d]
control	0.015	6.03	96	ND	ND	3.9	ND

[a] Data expressed as percent of radioactivity recovered from silica gel fractions. [b] R_f values refer to LQ6DF plates developed with the upper phase of benzene-acetic acid-water (6:7:3) with the following compounds: R_f 0.05 = 4-O-sulfopropylhesperetin dihydrochalcone; R_f 0.20 = 3-(3,4-dihydroxyphenyl)propanoic acid; R_f 0.47 = 3-(3-hydroxyphenyl)propanoic acid; R_f 0.60 = 3-(3-hydroxy-4-methoxyphenyl)propanoic acid. [c] ND = none detected. [d] Unidentified.

Table IV. Chromatographic Distribution of Radioactivity in the Urine of Rats Orally Dosed with 4-O-Sulfopropylhesperetin Dihydrochalcone-β-^{14}C

	% of total radioactivity[a] on TLC plate: remaining at origin	R_f 0.15	R_f 0.35	R_f 0.62	R_f 0.72
before enzymatic deconjugation	100	ND[b]	ND	ND	ND
after enzymatic deconjugation	75[c]	25	ND	ND	ND

[a] R_f values refer to PQ5F 1000 plates developed with chloroform-methanol-acetic acid (18:4:1) with the following compounds: R_f 0.15 = 4-O-sulfopropylhesperetin dihydrochalcone; R_f 0.35 = 3-(3,4-dihydroxyphenyl)propanoic acid; R_f 0.62 = 3-(3-hydroxyphenyl)propanoic acid; R_f 0.72 = 3-(3-hydroxy-4-methoxyphenyl)propanoic acid. [b] ND = not detected. [c] Metabolites at origin unidentified.

sulfonic acid) exhibited intestinal absorption of less than 1.2% of the administered dose. This latter figure is nearly two orders of magnitude less than the parent hesperetin-3-^{14}C compound.

The stability of 4-O-sulfopropylhesperetin dihydrochalcone-β-^{14}C to rat gut microflora in vitro is indicated by the data in Table III. The chromatographic distribution of radioactivity in the urine of rats orally dosed with 4 is shown in Table IV. While the majority of the radioactivity excreted in the urine remained unidentified, none of the usual phenylpropanoic acids were detected.

DISCUSSION

There have been relatively few studies of mammalian intestinal absorption using a related series of agents with increasing molecular weight. Hathway showed that intestinal absorption of a series of substituted phenolic antioxidants, the Ionox series, was inversely related to molecular weight (1966). Ionox 330 [2,4,6-tri(3′,5-di-*tert*-butyl-4′-hydroxybenzyl)mesitylene], the highest molecular weight member of the series, was essentially nonabsorbed after oral administration to rats, dogs, and man (Wright et al., 1965). Chadwick et al. (1977), using a characterized mixture of low-molecular-weight polyethylene glycols (PEG400, molecular weight range 232 to 594), found a similar inverse relation with about 10% of the highest molecular weight PEG member absorbed and excreted in the urine. A recent study by Parkinson et al., dealing with the intestinal absorption of polymeric antioxidant (peak molecular weight 4500) and related dimer (mol wt 270), trimer (mol wt 380), and tetramer–pentamer (mol wt 600) fractions, showed a molecular weight absorption limit of about 600–700 for these divinylbenzene–hydroquinone phenolic condensation products in the rat (1978). This latter study, like the present, employed bile duct ligated animals for a more accurate assessment of intestinal absorption particularly where absorbed agents are likely to undergo biliary excretion and enterohepatic circulation (Halladay et al., 1978).

The metabolic fate of hesperetin-3-^{14}C and the synthesis and sensory evaluation of a number of hesperetin DHC sweeteners have been described in previous publications from these laboratories (Honohan et al., 1976; DuBois et

al., 1977b). The results of this report demonstrate that intestinal absorption of hesperetin derivatives can be reduced by nearly two orders of magnitude by increasing molecular weight and charge through *O*-sulfoalkylation and "dimerization". The findings also prove that absorption levels of approximately 1% can be obtained with nonpolymeric, water-soluble derivatives with molecular weights of about 1000 and charged functional groups. The data in Table II also demonstrate, indirectly, the nearly complete stability of the O-sulfoalkylated hesperetin derivatives toward the intestinal microflora metabolism typical of flavonoid compounds and previously demonstrated for hesperetin-3-^{14}C (Honohan et al., 1976) and neohesperidin DHC (Gumbmann et al., 1976). In the case of the 4-*O*-sulfopropylhesperetin dihydrochalcone-β-^{14}C, stability was demonstrated by in vitro anaerobic incubations with rat cecal microflora (Table III) and by the absence of ^{14}C-labeled phenylpropanoic acids in the urines of orally dosed animals (Table IV). It seems clear that the higher molecular weight DHC derivatives are also highly resistant to microbial degradation.

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Gas Chromatographic–Mass Spectrometric Investigation of Hop Aroma Constituents in Beer

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More than 110 aroma constituents of a German beer, which delivered a desirable, fine hops aroma, were identified and semiquantified by means of liquid–liquid extraction, liquid–solid chromatography, and capillary gas chromatography–mass spectrometry. Forty-five constituents (among them: esters, ketones, alcohols, ethers, terpenoids, and sesquiterpenoids) were characterized for the first time in beer. Forty-seven of the determined volatiles had been found in Spalter hops.

Many beer consumers prefer beers with a distinct hops flavor, whereby, in addition to hops bitter, a fine hops aroma is also desirable. The bitter character of beer is essentially determined by the isomerization of humulone into isohumulone. At the present there are few analytical reference points as to what extent the hops aroma constituents are transferred to beer. To date more than 150 constituents of the essential oil of hops are known (van Straten and de Vrijer, 1973); however, there are few indices as to the extent of their contribution of beer flavor. The presence of the major sesquiterpenes of hop oil could not be proved by the use of presently available techniques. This led to the assumption that oxygenated hop oil constituents which have sensory relevance are also not present in beer.

In 1967 Buttery and Black identified ethyl dec-4-enoate, ethyl deca-4,8-dienoate, 2-heptanone, and ethyl heptanoate

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