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.

ACKNOWLEDGMENT

We thank the North Carolina State University, Raleigh, and the U.S. Department of Agriculture, Oxford Tobacco Research Station, Oxford, for growing and curing various tobacco used in making cigarettes used in these studies, P. Lorillard Co., Greensboro, and R. J. Reynolds Tobacco Co., Winston-Salem, for making cigarettes from the above-mentioned tobaccos, Neil A. Thaggard of P. Lorillard, Co., Greensboro, for helping us in standardizing our smoking machine, and Uniroyal Chemical Co. for supplying the MH and K-MH.

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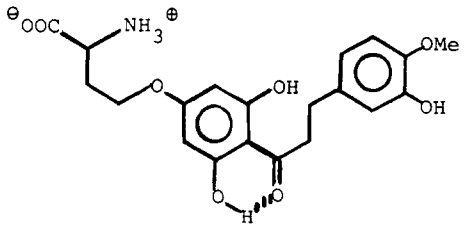
Received for review April 20, 1981. Revised manuscript received October 26, 1981. Accepted March 15, 1982. These studies were supported by U.S. Department of Agriculture, SEA/CR, Research Grant 801-15-16-B. Presented at the 7th International Tobacco Scientific Congress, Manila, Philippines, Nov 10, 1980.

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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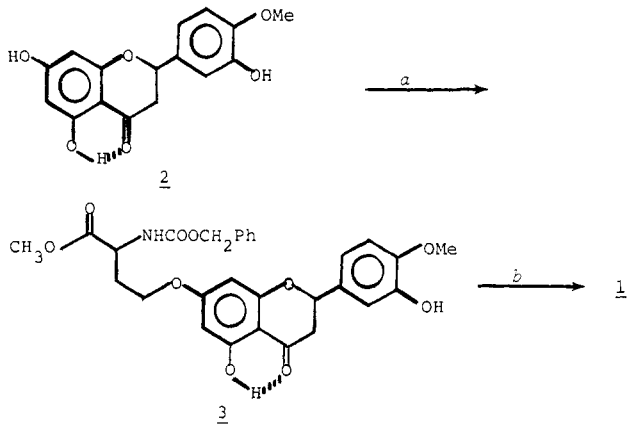
Scheme I



[a] K_2CO_3-NMP-$XCH_2CH_2CH(NHCOOCH_2Ph)COOCH_3$. [b] 5% Pd/C-10% KOH.

enantiomerically pure amino acids have solubilities substantially greater than those the corresponding racemic mixtures (Weast, 1969). Thus, in the interest of improving the solubility of 1, we decided to prepare 1 in the optically pure D and L forms. Additionally, it was of interest to ascertain information on the potency of the D and L forms of 1. Clearly, if only one antipode was responsible for the potency of DL-1 [HCl: (380 ± 40)× sucrose] and the other was tasteless, the sweet antipode would be twice as potent (760× sucrose) as the racemic material. This would allow for a halving of the concentration required in food systems and that, coupled with a solubility improvement expected for the sweet antipode, may substantially improve the utility of 1.

SENSORY EVALUATION

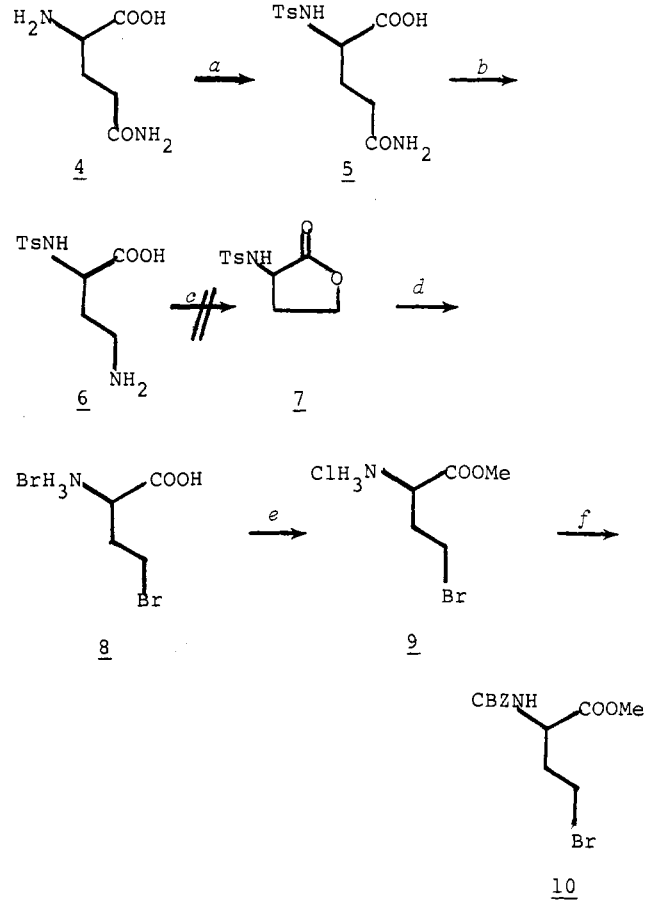
The two experimental compounds, which are described below, were evaluated by a human sensory panel. The same criteria, regarding purity, absence of acute toxicity, and bacterial mutagenicity, that were applied in our earlier work (DuBois et al., 1981b) were applied to these materials. Since both compounds passed these tests, they were subjected to sensory analysis by a trained panel of judges. Panelists were required to carry out magnitude estimation [vs. 0.25 M (8.55%) sucrose] and taste quality (percent sweet, sour, salty, bitter, and other) evaluations in one sensory session. From this analysis comparative taste potency data, calculated on both weight (P_w) and molar (P_m) bases, and taste quality data were obtained. All of the sensory methods which were employed for the present study are described in complete detail in our earlier work (DuBois et al., 1981b); the parameters I_w and I_m are equivalent to P_w and P_m, respectively, in the present work.

RESULTS AND DISCUSSION

For synthesis of the D and L antipodes of 1 by the method that we have described earlier (DuBois et al., 1981a), we required the corresponding D and L alkylating agents D-10 and L-10 to be used in alkylating hesperetin (2) as shown in Scheme I.

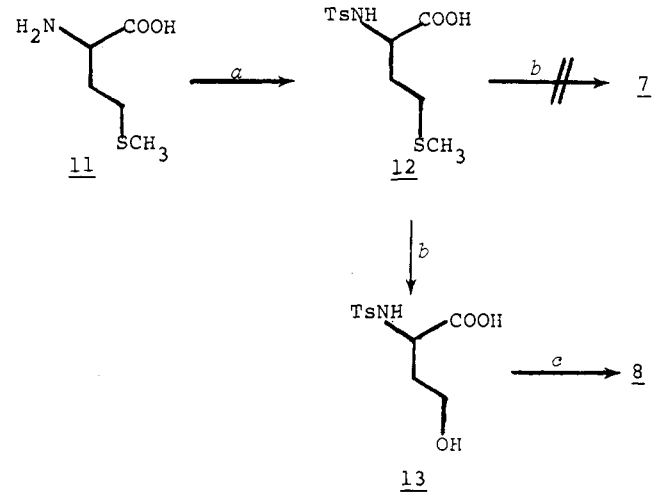
Several years ago, Rudinger and co-workers (Rudinger et al., 1959, 1960; Rudinger and Jost, 1967) reported preparation of L-10 from L-glutamine (4) as illustrated in Scheme II. Unfortunately, whereas conversion of L-4 to L-6 proceeded as expected, we were completely unsuccessful in the diazonium ion mediated conversion of L-6 to L-7. More recently Sugano and Miyoshi (1973) reported preparation of L-7 from L-methionine (11) as illustrated in Scheme III. In our hands, whereas preparation of 12 proceeded as expected, the published procedure for con-

Scheme II



[a] TsCl-Et_2O-NaOH. [b] NaOH-Br_2. [c] $NaNO_2$-HCl. [d] HBr-HOAc. [e] HCl-CH_3OH. [f] $NaHCO_3$-CbzCl.

Scheme III



[a] TsCl-Et_2O-NaOH. [b] CH_3I-HOAc-HCOOH-H_2O. [c] HBr-HOAc.

version of L-12 to L-7 gave no significant amount of lactone product. Instead, a 71% yield of the crystalline hydroxy acid L-13 was obtained. Treatment of L-13, however, under the L-7 ← L-8 conditions of Rudinger and Jost gave L-8 in 95% yield. Conversion of L-8 to L-10 then proceeded as was reported. Thus, L-10 was obtained in 49% overall yield from L-methionine.

Bromide L-10 was converted to the substantially more reactive iodide (NaI-Me_2CO, 4 h, 20 °C), immediately prior to alkylation of hesperetin. The expected substitution product L-3 was then obtained in 72% yield after which

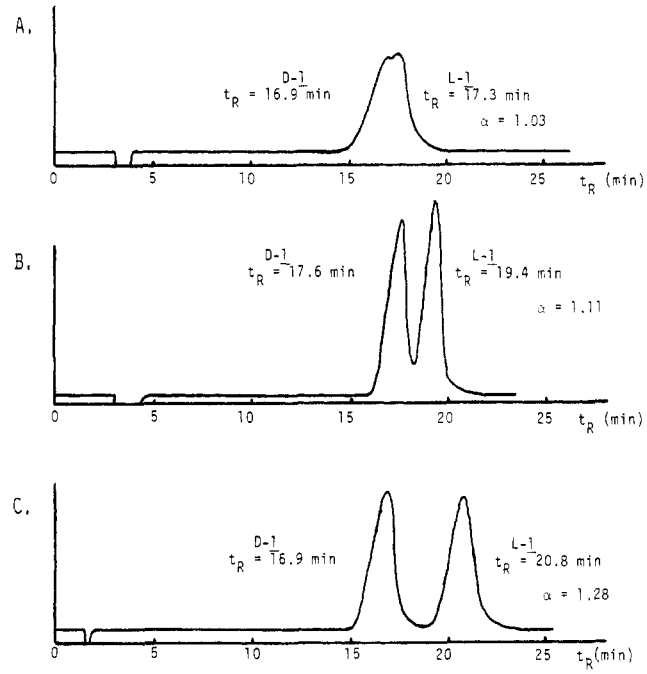


Figure 1. Chiral eluant high-performance liquid chromatography of DL-2,3',6-trihydroxy-4-(3-amino-3-carboxypropoxy)-4'-methoxydihydrochalcone. (A) Eluant: 50:50 0.016 M $Cu(OAc)_2$–0.034 M L-Pro–CH_3OH. (B) Eluant: 50:50 0.016 M $Cu(OAc)_2$–0.034 M L-Val–CH_3OH. (C) Eluant: 50:50 0.001 M $Cu(OAc)_2$–0.001 M L-Asp-L-Phe-OMe–CH_3OH. Separation factors α were calculated from the relationship $\alpha = [(t_R)_2 - t_0]/[(t_R)_1 - t_0]$ where $(t_R)_2$ and $(t_R)_1$ are the retention times of the L and D antipodes, respectively, and t_0 is the time for elution of the column void volume.

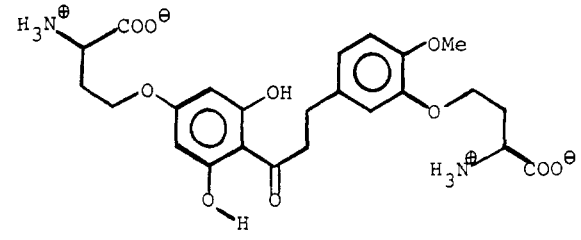
hydrogenation in alkali gave L-1 quantitatively. The free amino acid L-1 was then converted to the highly water soluble hydrochloride salt L-1·HCl by the method described for DL-1 (DuBois et al., 1981a). Thus, L-1·HCl was obtained in 56% overall yield from **2**.

Preparation of D-1·HCl, which involved preparation of D-**10** from D-methionine, proceeded in an exactly analogous manner to that described above for L-1·HCl.

Prior to investigation of the solubility and sensory properties of the D-1 and L-1 prepared above, it was considered essential to ascertain the enantiomeric purity of the samples. Recently, Hare and Gil-Av (1979) published results documenting their discovery of a very powerful chromatographic method for the determination of the enantiomeric purity of α-amino acids. Using an ion-exchange HPLC column, these workers achieved separation of 18 racemic amino acids by elution with aqueous 0.008 M $CuSO_4$–0.016 M L-proline. The optical antipodes are separated by virtue of differing diasteromeric nonbonded interactions that occur during ligand exchange with the chiral Cu(II)–L-Pro complex. Primary amino acids were then detected fluorometrically following postcolumn derivatization with *o*-phthalaldehyde. We found that similar separations could be achieved on a commercially available reverse-phase HPLC column. Chiral eluant chromatography of DL-1 was simplified since the strong UV chromophore (λ_{max} 284 nm, ϵ 14 500) allows detection without derivatization. Thus in analysis of DL-1 with Cu(II)–L-Pro as the chiral eluant, detectable (α = 1.03) separation (Figure 1A) was achieved. Unfortunately, this was not sufficient for enantiomeric purity assessment. UV detection, where applicable, renders the chiral eluant method to be very powerful, since virtually any α-amino acid may be chosen as the chiral species. In the fluorometric method of Hare and Gil-Av (1979), the chiral species must be a secondary amino acid to avoid reaction with *o*-phthalaldehyde. Thus, various primary α-amino acids were tried as chiral species. L-Valine (Figure 1B) gave particularly good results (α = 1.11), which allowed us to determine the enantiomeric purity of D-1 and L-1. Both synthetic products were found to be ≥99.5% enantiomerically pure. The power and utility of this method, where applicable, cannot be overemphasized, since enantiomeric purity can be determined in a totally unambiguous manner. This is a great improvement over the classical method of recyrstallization to maximum optical rotation where enantiomeric purity is never known with confidence.

Shortly after the report of Hare and Gil-Av, Grushka et al. (1979) reported excellent separations of several aromatic α-amino acids on a reverse-phase HPLC column by use of a chiral eluant consisting of a copper(II) complex of the dipeptide sweetener Aspartame (L-Asp-L-Phe-OMe). When a modification of these conditions is employed, excellent separation (α = 1.28) of DL-1 was achieved (Figure 1C). As separation of DL-1 may be achieved at much lower copper(II) concentrations, background absorption is reduced and sensitivity much increased such that detection is possible at the absorption maxima of **1**. Thus, the Cu(II)-L-Asp-L-Phe-OMe system is preferred for analysis of **1**.

In a carefully controlled study, the solubilities of DL-1 and L-1 were determined. At 22 °C and pH 3.6, DL-1 was found to have a solubility of 18 mg/L and L-1 a solubility of 33 mg/L. Thus, L-1 was found to have solubility 1.8× that of racemic **1**. The solubility of DL-1 determined in this study was significantly different from the value of 45.3 mg/L measured earlier (DuBois et al., 1981a). Further investigation showed that the sample used for the earlier study contained a small amount (ca. 1%) of an impurity **14**. Subsequently, this biszwitterionic compound was



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found to have a very pronounced effect on the stability of DL-1 solutions.

Sensory evaluation vs. 8.55% sucrose (0.25 M) of D-1 and L-1 was then carried out as described above. The results of these evaluations as well as comparative data from DL-1 (DuBois et al., 1981a) are summarized in Table I. From this data it is clear that both D and L enantiomers of **1** have sweetness potencies and qualities that are not significantly different from those of DL-1. Thus, neither antipode exhibits increased potency so as to allow concentration reduction in food systems. However, an increase in solubility is observed for the enantiomers over racemic **1**. Therefore, the utility of L-1 in food systems is expected to be significantly greater than that of the less soluble racemic compound.

EXPERIMENTAL SECTION

Synthetic Procedures. All organic starting materials and reactants were obtained from Aldrich Chemical Co. except for hesperetin, D-methionine, and L-methionine, which were obtained from Sigma Chemical Co. All inorganic reagents were obtained from J. T. Baker Chemical Co. except for 5% palladium on carbon (Pd/C) hydrogenation catalyst (Engelhard Minerals + Chemicals Corp.)

Table I. Sensory Analysis of DL-, D-, and L-2,3',6-Trihydroxy-4-(3-amino-3-carboxypropoxy)-4'-methoxydihydrochalcone Hydrochloride[a]

compd	judgments	concn, ppm	I_p	P_w	P_m	taste character: sweet	sour	salty	bitter	other	sweet:bitter
DL-1·HCl	16	225	1.0 (0.1)	380 (40)	490 (50)	85 (6)	2 (1)	0 (0)	5 (4)	8 (4)	94:6
D-1·HCl	12	220	1.0 (0.1)	390 (40)	510 (50)	86 (8)	2 (2)	0 (0)	6 (6)	6 (8)	93:7
L-1·HCl	12	235	0.9 (0.1)	330 (30)	420 (50)	84 (7)	3 (2)	0 (0)	5 (3)	8 (2)	94:6

[a] Sensory data are reported as follows: mean value ($2S_m$); statistical calculations were carried out as described by Gordon and Ford (1972).

and anhydrous HBr and anhydrous HCl, which were obtained from Matheson Gas Corp. Solvents used were reagent grade and obtained from either J. T. Baker Chemical Co. or Fisher Scientific Co. *N*-Methyl-2-pyrrolidinone (NMP) was obtained from Aldrich Chemical Co. and was distilled from CaH_2 and stored over activated (400 °C, 3 h) molecular sieves, 3A (J. T. Baker Chemical Co.) prior to use.

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 137 infrared spectrometer and proton magnetic resonance spectra (NMR) on a Varian T-60A spectrometer and are reported as parts per million (ppm) relative to tetramethylsilane. Combustion analyses were performed by the Microanalysis Laboratory, Stanford University, Stanford, CA. Optical rotations were determined on a Perkin-Elmer Model 431 polarimeter.

Analytical thin-layer chromatography (TLC) was carried out on prelayered silica gel F-254 plates (E. Merck, Darmstadt, West Germany), visualizing with either UV light or iodine staining. Column chromatography was carried out on 60–200-mesh silica gel powder (J. T. Baker Chemical Co.).

High-pressure liquid chromatography (HPLC) was performed on a Waters Associates instrument equipped with a Model 660 solvent programmer and two Model 6000A pumps. Analytical work was carried out on a μBondapak C-18 reverse-phase column (30 cm × 3.9 mm i.d.) eluting with a linear program (15 min; 2 mL/min) of 10–100% CH_3OH in 0.03 M KH_2PO_4 buffer. The detector employed was a Schoeffels Model SF 770 spectroflow monitor equipped with a Model GM 770 monochromator. Chiral eluant chromatography was carried out on the same column while eluting at a rate of 1.0 mL/min with a 50:50 mixture of CH_3OH and either (1) 0.016 M $Cu(OAc)_2$–0.034 M L-proline [Cu(II)–L-Pro system], (2) 0.016 M $Cu(OAc)_2$–0.034 M L-valine [Cu(II)–L-Val system], or (3) 0.001 M $Cu(OAc)_2$–0.001 M aspartame [Cu(II)–L-Asp-L-Phe-OMe system]. The detector reference cell was filled with the eluant in use. Detection was carried out at 310 nm [Cu(II)–L-Pro and Cu(II)–L-Val systems] and 286 nm (Cu(II)–L-Asp-L-Phe-OMe system].

Unless otherwise indicated, all reactions were carried out under an inert atmosphere of argon at ambient temperature with vigorous stirring, magnetic for homogeneous and overhead for nonhomogeneous reactions, and all reagents employed were anhydrous. Standard workup of reactions employing H_2O-immiscible solvents (e.g., Et_2O, EtOAc, etc.) involved addition of H_2O, neutralization with aqueous acid or base, extraction of the mixture with an appropriate organic solvent (Et_2O, EtOAc, etc.), drying the combined extracts over $MgSO_4$, and concentration in vacuo. Standard workup of reactions employing NMP solvent involved dilution with H_2O, neutralization, extraction of the mixture with an appropriate solvent (Et_2O, EtOAc, etc.), washing the combined extracts with H_2O (6×), drying over $MgSO_4$, and concentration in vacuo. Hydrogenation reactions were carried out on a Parr hydrogenation apparatus. Standard workup involved filtration through Celite and neutralization with 10% HCl, followed by product isolation by filtration on a Büchner funnel.

The amino acids or mineral acid salts thereof were assayed with regard to purity by a combination of (1) proton titration, (2) HPLC, and (3) Karl Fischer analyses. Titrations were carried out with a Brinkman Metrohm Herisau Potentiograph E576.

L-Methyl 2-(Benzyloxycarbonyl)amido-4-bromobutanoate (L-10). According to the general procedure of Rudinger and co-workers (Rudinger et al., 1959, 1960; Rudinger and Jost, 1967), 14.9 g (0.10 mol) of L-methionine, as a solution in 120 mL of 1.00 N NaOH, was treated with 21.0 g (0.11 mol) of tosyl chloride as a solution in 50 mL of ether. The resultant heterogeneous reaction mixture was stirred vigorously at ambient temperature. Additional 1.00 N NaOH (100 mL) was added dropwise over 2 h at such a rate as to maintain a pH of 9.2. The reaction mixture was then acidified to pH 1 with 10% HCl after which standard workup yielded 33.5 g of crude tosyl-L-methionine (L-12) as a colorless oil: TLC (toluene–dioxane–HOAc, 4.5:4.5:1.0) R_f 0.58.

The crude tosylamide L-12 was treated with 25.1 g (0.18 mol) methyl iodide as a solution in 30 mL acetic acid, 48 mL of formic acid, and 12 mL of water according to the procedure of Sugano and Miyoshi (1973). After 13 h, workup yielded a dark oil which was dissolved in sufficient 1.00 N NaOH to yield a solution of pH 6. The resultant reaction mixture was heated to 90 °C for 3 h during which time 1.00 N NaOH was added in portions so as to maintain a pH of 6–7. When the mixture was cooled to ambient temperature, followed by being cooled in an ice bath, no crystals or oil separated as had been reported. The pH was then adjusted to 1 by addition of 10% HCl, causing a large amount of precipitation. The precipitate was filtered on a Büchner funnel to give 13.2 g of an off-white solid. The filtrate was saturated with NaCl and extracted with EtOAc (3 × 50 mL) to give 9.8 g of additional off-white solid. Recrystallization (EtOAc–hexane) of these two combined products yielded 19.4 g of tosyl-L-homoserine (L-13) as colorless clusters: mp 124–126 °C; IR (KBr) 2.91 (O–H), 3.05 (N–H), 5.82 (C=O), 7.69 (S=O), 8.68 (S=O) μm; NMR (Me_2CO) δ 1.70–2.17 (m, 2 H, O–C–CH_2–C–COO), 2.40 (s, 3 H, Ar–CH_3), 3.66 (t, 2 H, J = 6 Hz, O–CH_2), 3.87–4.31 (m, 1 H, CH–N), 6.53 (br abs, 3 H, COOH, OH, NH), 7.34 (d, 2 H, J = 7 Hz, 3,5-ArH), 7.76 (d, 2 H, J = 7 Hz, 2,6-ArH). Tosyl-L-homoserine (L-13) was converted to 2-amino-4-bromobutanoic acid hydrobromide (L-8) by a modification of the procedure of Rudinger and Jost (1967). Thus 19.4 g (0.0709 mol) of L-13 was dissolved in 180 mL of 35% HBr–HOAc in a 300-mL Fisher-Porter pressure bottle. The resultant reaction mixture was heated at 75–80 °C for 5 h, was allowed to cool, and was then concentrated in vacuo to yield an orange solid. The solid product was triturated with Et_2O, filtered on a Büchner

funnel, and dried in vacuo to yield 17.7 g (95%) of L-8 as a white flocculent solid: mp 176–184 °C with decomposition [lit. (Rudinger and Jost, 1967) mp 188–190 °C]. According to the procedure of Rudinger and Jost (1967), 17.5 g (0.0666 mol) of carboxylic acid L-8 was treated with anhydrous HCl in 250 mL of absolute methanol to yield 18.7 g of methyl L-2-amino-4-bromobutanoate hydrochloride (L-9) as an oily solid. Crude L-9 was then dissolved in 90 mL of H_2O after which the solution obtained was cooled to 0 °C. While the solution was stirred vigorously, 12.3 g (0.146 mol) of sodium bicarbonate was added in one portion followed immediately by 11.4 mL (13.6 g, 0.0799 mol) of benzylchloroformate, also in one portion. Stirring at 0 °C was continued for 30 min after which the reaction mixture was allowed to warm to ambient temperature over 2 h. Standard workup with Et_2O then yielded 23.7 g of crude L-10 as a mixture of oil and crystals. Trituration of this mixture (Et_2O–hexane) yielded 3.82 g of colorless tiny clusters: mp 88–90 °C [lit. (Rudinger and Jost, 1967) mp of L-10 62–64 °C; lit. (DuBois et al., 1981a) mp of DL-10 87–89 °C]; $[\alpha]^{20}_D$ 1.2° (*c* 0.50, DMF) [lit. (Rudinger and Jost, 1967) $[\alpha]_D$ −41.2° (*c* 0.5, DMF)]. The residual oil was then chromatographed over 200 g of silica gel (hexane–EtOAc) to yield 16.1 g (49% overall from L-methionine) of L-10 as a viscous oil. Crystallization (hexane–Et_2O) yielded 9.62 g of tiny colorless clusters: mp 61–63 °C [lit. (Rudinger and Jost, 1967) mp 62–63 °C]; $[\alpha]^{20}_D$ −40.6° (*c* 0.512, DMF) [lit. (Rudinger and Jost, 1967) $[\alpha]_D$ −41.2° (*c* 0.5; DMF)]; TLC (hexane–EtOAc, 1:1) R_f 0.40; IR (KBr) 3.04 (N–H), 5.80 (ester C=O), 5.96 (urethane C=O) μm; NMR ($CDCl_3$) δ 2.04–2.56 (m, 2 H, Br–C–CH_2), 3.40 (t, 2 H, *J* = 6 Hz, $BrCH_2$), 3.74 (s, 3 H, $COOCH_3$), 4.40–4.63 (m, 1 H, CH–N), 5.12 (s, 2H, $PhCH_2$), 5.50 (d, 1 H, *J* = 7 Hz, N–H), 7.34 (s, 5 H, PhH). Anal. ($C_{13}H_{16}BrNO_4$) C, H.

L-2,3′,6-Trihydroxy-4-(3-amino-3-carboxypropoxy)-4′-methoxydihydrochalcone Hydrochloride (L-1·HCl). To a solution of 16.5 g (0.050 mol) of L-10 in 250 mL of acetone was added 15.0 g (0.10 mol) of NaI. After the mixture was stirred at ambient temperature for 4 h, HPLC analysis (60% MeOH in 0.03 M KH_2PO_4; 254 nm) showed all L-10 (t_R = 5.0 min) to have been consumed to yield one product (t_R = 6.4 min). The NaBr was then filtered and the acetone removed in vacuo after which standard ether workup yielded 18.9 g (100%) of L-methyl 2-(benzyloxycarbonyl)amido-4-iodobutanoate (L-15) as a light yellow solid.

The crude iodide L-15 (18.9 g, 0.050 mol), 7.60 g (0.055 mol) of K_2CO_3, and 15.1 g (0.050 mol) of hesperetin were then reacted as a solution in 150 mL of NMP for 64 h at ambient temperature by the general method which we have described earlier (DuBois et al., 1981a). Standard workup (EtOAc) followed by chromatography over 600 g of silica gel ($CHCl_3$–CH_3OH) yielded 24.8 g of a 96:4 mixture of the desired monoalkylation product L-3′,5-dihydroxy-4′-methoxy-7-[3-carbomethoxy-3-[(benzyloxycarbonyl)-amido]propoxy]flavanone (L-3), and the 3′,7-dialkylation product. Quantitative HPLC analysis (75% CH_3OH in 0.03 M KH_2PO_4; 286 nm) indicated a 72% yield (19.8 g) of L-3.

The purified flavanone L-3 was dissolved in 75 mL of THF and added dropwise with stirring at 0 °C to 150 mL of 10% KOH. After the solution was flushed with argon, 5.0 g of 5% Pd/C was added and the resultant reaction mixture hydrogenated on a Parr hydrogenation apparatus at 35 psi H_2 pressure for 6.5 h. The dark reaction mixture was then filtered through Celite, acidified to pH 5.9 with 10% HCl, and concentrated in vacuo to ca. 100 mL. After the mixture was allowed to stand overnight, filtration on a Büchner funnel, followed by air-drying, yielded 15.0 g of L-2,3′,6-trihydroxy-4-(3-amino-3-carboxypropoxy)-4′-methoxydihydrochalcone (L-1) as a light tan solid: TLC (toluene–dioxane–HOAc; 4.0:4.0:2.0) R_f 0.06; HPLC (linear 15 min 10–100% CH_3OH in 0.03 M KH_2PO_4 program; 286 nm) t_R = 11.7 min. L-1 (15.0 g) was then dissolved with heating in 700 mL of 2 N HCl. The resultant solution was decolorized with 5 g of Norit, filtered through Celite, allowed to cool slowly to ambient temperature, and then cooled to 0 °C for 16 h. Filtration on a Büchner funnel, followed by washing with several portions of ice-cold 1 N HCl, air-drying, and drying in a vacuum desiccator (100 mmHg) over P_2O_5 for 3 days, yielded 12.5 g of (56% overall from hesperetin) L-1·HCl as colorless needles: mp 95–190 °C dec; $[\alpha]^{20}_D$ +14.0° (*c* 0.751, DMF). Anal. ($C_{20}H_{23}NO_8$·HCl·H_2O) C, H, N. Proton titration indicated this product to be 85.1% free amino acid L-1 and 8.1% HCl. Karl Fischer titration showed the presence of 7.6% water. Chiral eluant chromatography [Cu(II)–L-Asp-L-Phe-OMe system] showed only L-1 (t_R = 20.8 min) to be present. No D-1 (t_R = 16.9 min) was found to a detection limit of 0.5%, thus showing the product obtained to be enantiomerically pure.

D-Methyl 2-(Benzyloxycarbonyl)amido-4-bromobutanoate (D-10). According to procedures identical with those employed above for the preparation of L-10, 14.9 g (0.10 mol) of D-methionine was processed to yield 7.92 g (24% overall) of D-10: mp 58–62 °C [lit. (Rudinger and Jost, 1967) mp of L isomer 62–64 °C]; $[\alpha]^{20}_D$ +39.3° (*c* 0.51, DMF) [lit. (Rudinger and Jost, 1967) $[\alpha]_D$ = −41.2° (*c* 0.5, DMF) for L isomer].

D-2,3′,6-Trihydroxy-4-(3-amino-3-carboxypropoxy)-4′-methoxydihydrochalcone Hydrochloride (D-1·HCl). D-10 (1.65 g, 5.00 mmol) was converted to D-methyl 2-(benzyloxycarbonyl)amido-4-iodobutanoate (D-15) in quantitative yield by reaction with NaI (1.50 g, 10 mmol) in 25 mL of acetone as reported above for L-10.

The crude iodide D-15 (1.89 g, 5.00 mmol) was reacted with 1.51 g (5.00 mmol) of hesperetin and 0.76 g (5.50 mmol) of K_2CO_3 in 15 mL of NMP by the general procedure which we have described earlier. Standard workup (EtOAc) followed by chromatography over 200 g of silica gel ($CHCl_3$–CH_3OH) yielded 2.63 g of a 94:6 ratio of the desired monoalkylation product, D-3′,5-dihydroxy-4′-methoxy-7-[3-carbomethoxy-3-[(benzyloxycarbonyl)-amido]propoxy]flavanone (D-3), and the 3′,7-dialkylation product. Quantitative HPLC analysis (75% CH_3OH in 0.03 M KH_2PO_4; 286 nm) indicated a 58% yield (1.61 g) of D-3.

By the procedure described above for hydrogenation of L-3, 1.61 g (2.91 mmol) of D-3 was hydrogenated over 0.40 g of 5% Pd/C as a solution in a mixture of 10 mL of THF and 50 mL of 10% KOH to yield 1.18 g (100%) of D-2,3′,6-trihydroxy-4-(3-amino-3-carboxypropoxy)-4′-methoxydihydrochalcone (D-1) as a light tan solid: TLC (toluene–dioxane–HOAc, 4.0:4.0:2.0) R_f 0.06; HPLC (linear 15 min 10–100% CH_3OH in 0.03 M KH_2PO_4 program; 286 nm) t_R = 11.7 min. D-1 (1.18 g, 2.91 mmol) was converted to the hydrochloride salt, as described above for L-1, by dissolution in 80 mL of 1 N HCl to yield, after drying only, 0.84 g (38% overall from hesperetin) of D-1·HCl as colorless needles; mp 95–190 °C dec; $[\alpha]^{20}_D$ −15.7° (*c* 0.757, DMF). Anal. ($C_{20}H_{23}NO_8$·HCl·1.5H_2O) C, H, N. Proton titration indicated this product to be 79.5% free amino acid D-1 and 7.7% HCl. Karl Fischer titration showed the presence of 14.0% water. Chiral eluant chromatography [Cu(II)–L-Asp-L-Phe-OMe system] showed only D-1 (t_R = 16.9 min) to be present. No L-1 (t_R = 20.8 min) was found to a

detection limit of 0.5%, thus showing the product to be enantiomerically pure.

Solubility Determination. Ten milliliters of hot (65 ± 2 °C) distilled water was rapidly added to 10.2 mg of dry L-1·HCl while rapidly magnetically stirring in a 50-mL beaker. The homogeneous solution thus obtained was diluted with hot water to 50 mL in a warm volumetric flask. This hot solution was filtered through a 0.45-μm Type HA Millipore filter into a warm 50-mL volumetric flask. The concentration of L-1·HCl in the filtrate, which was maintained at 65 ± 2 °C, was then determined by HPLC analysis (2.0 mL/min 60% CH_3OH in 0.03 M KH_2PO_4; 286 nm) to be 191 mg/L. No crystallization or precipitation of L-1 was observed on cooling. After 4 h, HPLC analysis indicated the concentration of L-1·HCl to be 183 mg/L. Crystallization of L-1 then began to occur slowly. After 94 h (22 °C), the concentration of L-1 was determined to be 33 mg/L. The pH of this solution was 3.65.

An identical procedure employing 10.3 mg of DL-1·HCl yielded a solution having a DL-1·HCl concentration of 205 mg/L. After the solution was cooled over 4 h, the concentration had dropped to 186 mg/L, and after 94 h (22 °C) a concentration of 18 mg/L was determined. The pH of this solution was 3.57.

ACKNOWLEDGMENT

We thank G. McGarraugh and Dr. R. Phillips for analytical studies, P. Dietrich, F. Enderlin, Dr. J. Brown, and Dr. T. Parkinson for biological safety studies, J. Lee for sensory studies, and S. de Keczer and Dr. G. Crosby for useful discussions of this work.

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Received for review October 7, 1981. Accepted March 15, 1982.

Volatile Constituents of the Muscadine Grape (*Vitis rotundifolia*)

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The volatile constituents of the muscadine grape have been studied by the combined technique of gas chromatography and mass spectroscopy. Forty-nine compounds have been identified and confirmed. A gas chromatogram of a muscadine extract and a table of compounds and their associated mass spectral data are presented.

The muscadine grape grows abundantly throughout the southeastern United States. It is a large, dark, thick-skinned grape that ripens in late August and continues through September. The muscadine, which is cultivated and grows wild, can vary in flavor, color, and size, thereby resulting in several varieties of the grape. Because muscadines are rich in flavor, they are widely used by local residents to produce homemade foods and beverages (Wigginton, 1975).

Foods and beverages produced from muscadines have been studied extensively. The characteristics of muscadine wines (Carroll et al., 1975), preserves (Rizley et al., 1977), and juices and jellies (Flora, 1977a) have been reported. Additional research has included the cultivation (Lane, 1972), harvesting (Balerdi and Mortensen, 1973), processing (Flora, 1977b), and storage (Smit et al., 1971) of muscadine grapes.

Analytical research has included sugar and organic acid concentration (Carroll et al., 1971) as well as the relationship of anthocyanins to color (Nesbitt et al., 1974).

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Table I. Volatile Constituents of Muscadines Previously Reported

Kepner and Webb (1956)	
methyl alcohol	biacetyl
ethyl alcohol	1-hexanal
n-butyl alcohol	2-hexenal
isoamyl alcohol	ethyl acetate
1-hexanol	caproate ester
2-phenylethanol	caprylate ester
acetaldehyde	caprate ester
isobutyraldehyde	laurate ester
acetal[a]	methyl ethyl ketone[a]
Berry et al. (1979)	
methanol	ethyl acetate
ethanol	ethyl propionate
butanol	propyl acetate
2-methylbutanol	butyl acetate
hexanol	benzyl acetate
trans-2-hexen-1-ol	ethyl caprate
2-phenylethanol	*d*-limonene
trans-2-hexenal	

[a] Tentatively assigned.

The volatile constituents of the grape have been investigated by Kepner and Webb (1956) and Berry et al. (1979). These two investigations resulted in the identification of the major constituents of the grape. This paper presents

0021-8561/82/1430-0681$01.25/0