mass, IR, and ^1H NMR spectra and GLC retention data of the synthesized compound were identical with that of the unknown.

Very few of these types of compounds seem to have been reported in the literature. In the present work, for comparison, the homologue 2-butyl-3-methylmaleic anhydride was also synthesized by using the same procedure as that for the hexyl compound. 2-Isoamyl-3-methylmaleic anhydride had been synthesized by Auden et al. (1899) and 2-isobutyl-3-methylmaleic anhydride by Purdie and Holt (1965) using more involved synthetic procedures.

The spectral data for the 2-hexyl-3-methylmaleic anhydride are given below. The mass spectrum showed (two most intense ions each 14 mass units above m/e 34, intensities in parentheses, molecular ion in boldface) the following: 41 (33), 43 (50); 53 (12), 55 (14); 67 (12), 69 (9); 79 (4), 81 (10); 95 (12), 98 (25); 111 (4), 112 (6); 126 (100), 127 (9); 139 (9), 140 (13); 151 (3), 153 (4); 168 (6), 169 (1); **196** (3). High-resolution mass spectrometry molecular weight was found to be 196.1092 ($C_{11}H_{16}O_3$ requires 196.1099). The IR spectrum (film) showed absorption maxima (in microns between 5.0 and 16.0) as follows: strong (5.7, 7.8, 10.8, 13.5); medium (5.4, 5.5, 6.0, 6.8, 7.2, 8.9, 11.1); weak (8.2, 8.4, 9.7, 12.6, 14.6, 14.9). The ^1H NMR spectrum (90 MHz, $CDCl_3$, 34 °C) showed: δ 0.87 (t, 3 H, $-(CH_2)_5CH_3$), 1.28 (br s, 6 H, $-CH_2(CH_2)_3CH_3$), 1.55 (pt, 2 H, $=CCH_2CH_2-$) 2.05 (s, 3 H, $=CCH_3$), 2.44 (t, 2 H, $=CCH_2CH_2-$). The ^{13}C NMR spectrum (25.03 MHz, $CDCl_3$, 30 °C) showed δ 9.48 (q, 1 C, $CH_3C=$), 13.99 (q, 1 C, CH_3CH_2-), 22.46 (t, 1 C, CH_3CH_2-), 24.44 (t, 1 C, $-CH_2C=$), 27.55 (t, 1 C, $-CH_2CH_2C=$), 29.11 (t, 1 C, $-CH_2CH_2CH_2C=$), 31.36 (t, 1 C, $CH_3CH_2CH_2-$), 140.4 (s, 1 C, $-CH_2-C=$), 144.8 (s, 1 C, $CH_3C=$), 165.9 (s, 1 C, $-C=O$), 166.3 (s, 1 C, $-C=O$). The spectrum was compared with those of 2-methylmaleic anhydride and 1-octene as models (Johnson and Jankowski, 1972). The GLC Kovats retention index on the Pyrex Carbowax 20-M coated capillary was 2090. Carbon and hydrogen microanal. Calcd for $C_{11}H_{16}O_3$: C, 67.37; H, 8.22. Found: C, 67.1; H, 8.40.

The mass spectrum found for 2-butyl-3-methylmaleic anhydride showed the following: 41 (98), 43 (100); 53 (33), 55 (26); 67 (25), 69 (12); 79 (9), 81 (23); 95 (14), 98 (32); 111 (11), 112 (2); 125 (11), 126 (86); 139 (9), 140 (39); **168** (3).

Possible Mechanism of Formation and Role in Odor. We have been unable to come up with a likely mechanism for the formation of 2-hexyl-3-methylmaleic anhydride in raisins and almond hulls. We have, however, speculated on some remotely possible mechanisms. One of these is that methylmaleic anhydride is first formed in the dried fruits by dehydration of citric acid (this can be brought about in the laboratory) and then reacts with a hexyl free radical from lipid autoxidation. Another speculation is that the keto group of pyruvic acid ($CH_3COCOOH$) condenses with the α-methylene group in octanoic acid [$CH_3(CH_2)_5CH_2COOH$; present in raisins and almond hulls] and the dicarboxylic acid so formed dehydrates to the anhydride. Both of these postulated mechanisms would be difficult to bring about in the laboratory in high yield but might possibly be facilitated in the dried fruit by special conditions or special forms (or derivatives) of these elementary reactants.

In the author's opinion, the 2-hexyl-3-methylmaleic anhydride has very little odor and is unlikely to contribute to the aroma of raisins as far as humans are concerned. The olfactory senses of insects are, however, quite different from that of humans. The compound will be tested for attractancy with certain insects along with other raisin components in a study already under way with the Stored Product Insects Laboratory, USDA, Fresno, CA.

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An Intensely Sweet Analogue of Kynurenine: 3-(4-Chloroanthraniloyl)-DL-alanine

A new kynurenine derivative, 3-(4-chloroanthraniloyl)-DL-alanine, was synthesized from 6-chloro-DL-tryptophan. The former compound was found to be ~80 times sweeter than sucrose by organoleptic tests.

Excessive consumption of sugar has been implicated in several diseases such as diabetes, hyperlipidemia, obesity, cardiac infarction, and dental caries. Meanwhile, there is a continued uncertainty over the safety of well-known artificial sweeteners: cyclamate and saccharin. Thus, there is a great insistent need for a noncaloric, biologically safe sweetener.

A number of amino acids or their derivatives are known to have sweet taste (Ariyoshi, 1976). Although sweetness intensity of amino acids are generally weak, aromatic am-

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ino acids in the D configuration exhibit relatively intense sweet taste. Yamaguchi et al. (1970a,b) reported D-tryptophan is 25–50 times sweeter than sucrose. D-Tryptophan substituted in the 6 position by chlorine was found to be extremely much sweeter than unsubstituted D-tryptophan (Kornfeld et al., 1969). Finley and Friedman (1973) reported that the *N'*-acetyl derivative of kynurenine, a tryptophan metabolite, was ~35 times sweeter than sucrose.

From the above findings we expected that chlorination in the ring of kynurenine would give an intensely sweet compound. The purpose of the present paper is to describe the synthesis of DL-kynurenine substituted in the 4 position by chlorine and the sensory evaluation of its sweetness.

EXPERIMENTAL SECTION

Materials. 6-Chloro-DL-tryptophan was prepared from DL-tryptophan according to the method described by Moriya et al. (1975). Sucrose was obtained from a reliable commercial source.

Synthetic Procedure. All melting points are uncorrected. IR spectra were obtained on a Shimadzu IR-27G spectrophotometer. NMR spectra were recorded on a Hitachi R-20A instrument by using Me_4Si as an internal standard.

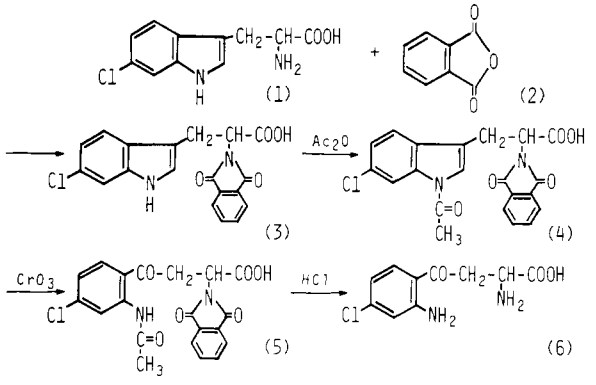
The synthesis of 3-(4-chloroanthraniloyl)-DL-alanine (4-chlorokynurenine) was carried out according to Ohki's method (Ohki and Nagasaka, 1971; Ohki, 1973) for the preparation of kynurenine from tryptophan.

***N*-Phthaloyl-6-chloro-DL-tryptophan (3).** A mixture of 6-chloro-DL-tryptophan (1) (39.0 g), phthalic anhydride (2) (26.0 g), and triethylamine (37 mL) in DMF (200 mL) was refluxed for 6 h. After evaporation of the solvent in vacuo, the residue was dissolved in ethyl acetate (300 mL). The solution was washed with 5% HCl, dried over $MgSO_4$, and evaporated to afford a solid product. Recrystallization of the solid from ethanol gave yellow prisms of *N*-phthaloyl-6-chloro-DL-tryptophan (3) (47.7 g, 79.2%): mp 259 °C dec; IR (KBr) ν_{max} 3420, 1755, 1710 cm^{-1}; NMR (Me_2SO-d_6) δ 3.54 (2 H, d, J = 8 Hz), 5.14 (1 H, t, J = 8 Hz), 6.90 (1 H, dd, J = 2 and 8 Hz), 7.06 (1 H, d, J = 3 Hz), 7.28 (1 H, d, J = 2 Hz), 7.51 (1 H, d, J = 8 Hz), 7.80 (4 H, s), 10.87 (1 H, br s). Anal. Calcd for $C_{19}H_{13}N_2O_4Cl$: C, 61.88; H, 3.55; N, 7.60; Cl, 9.61. Found: C, 61.53; H, 3.81; N, 7.72; Cl, 9.55.

***N*-Phthaloyl-1-acetyl-6-chloro-DL-tryptophan (4).** A mixture of 3 (45.4 g), sodium acetate (25 g), and acetic anhydride (500 mL) was refluxed for 6 h. After removal of acetic anhydride, H_2O was added to the residue and the mixture was shaken with chloroform. The solid product thus obtained was collected by filtration and recrystallized from methanol to afford colorless prisms of *N*-phthaloyl-1-acetyl-6-chloro-DL-tryptophan (4) (38.4 g, 75.9%): mp 240–240.5 °C; IR (KBr) ν_{max} 1780, 1720, 1670 cm^{-1}; NMR (Me_2SO-d_6) δ 2.49 (3 H, s), 3.2–3.6 (2 H, m), 5.1–5.3 (1 H, m), 7.23 (1 H, dd, J = 2 and 8 Hz), 7.65 (1 H, d, J = 8 Hz), 7.66 (1 H, s), 7.84 (4 H, s), 8.24 (1 H, d, J = 2 Hz). Anal. Calcd for $C_{21}H_{15}N_2O_5Cl$: C, 61.4; H, 3.68; N, 6.82; Cl, 8.63. Found: C, 61.1; H, 3.80; N, 6.97; Cl, 8.54.

***N*-Phthaloyl-3-(*N'*-acetyl-4-chloroanthraniloyl)-DL-alanine (5).** To a suspension of 4 (32.9 g) in acetic acid (600 mL) was added dropwise a solution of CrO_3 (24 g) in acetic acid (300 mL) at 10 °C with stirring. After being stirred overnight at room temperature, the reaction mixture was poured into ice water and extracted with chloroform. The chloroform extract was shaken with saturated $NaHCO_3$ solution. The aqueous layer was acidified with 10% HCl and then extracted with chloroform. After removal of the solvent, the resulting residue was recrystallized from ethanol to afford colorless needles of *N*-phthaloyl-3-(*N'*-acetyl-4-chloroanthraniloyl)-DL-alanine (5) (10.3 g, 31.0%): mp 224–227 °C dec; IR (KBr) ν_{max} 1775, 1720, 1650 cm^{-1}; NMR (Me_2SO-d_6) δ 2.03 (3 H, s), 3.4–4.3 (2 H, m), 5.2–5.5 (1 H, m), 7.25 (1 H, dd, J = 2 and 9 Hz), 7.90 (4 H, s), 7.95 (1 H, d, J = 9 Hz), 8.22 (1 H, d, J = 2 Hz), 10.9 (1 H, br s). Anal. Calcd for $C_{20}H_{15}N_2O_6Cl$: C, 57.91; H, 3,65; N, 6.75; Cl, 8.55. Found: C, 57.68: H, 3.79; N, 6.53; Cl, 8.37.

Scheme I. Synthetic Route of 3-(4-Chloroanthraniloyl)-DL-alanine



3-(4-Chloroanthraniloyl)-DL-alanine (6). A suspension of 5 (8.5 g) in 20% HCl (350 mL) was refluxed for 5 h. After the reaction mixture was cooled, a precipitated phthalic acid was filtered off. The filtrate was treated with charcoal and adjusted to pH 6.0 with NaOH. The precipitate thus obtained was recrystallized from 40% acetic acid to afford pale yellow needles of 3-(4-chloroanthraniloyl)-DL-alanine (6) (3.6 g, 72.4%): mp 223 °C dec; IR (KBr) ν_{max} 3430, 3280, 1600 (br) cm^{-1}; NMR (D_2O–NaOD) δ 3.72 (1 H, s), 6.63 (1 H, dd, J = 9 and 2 Hz), 6.76 (1 H, d, J = 2 Hz), 7.72 (1 H, d, J = 9 Hz). Anal. Calcd for $C_{10}H_{11}N_2O_3Cl$: C, 49.49; H, 4.57; N, 11.54; Cl, 14.61. Found: C, 49.20; H, 4.48; N, 11.32; Cl, 14.51.

Sensory Analyses. *Relative Sweetness Measurement.* Reference solutions of sucrose (2, 4, 6, and 8% w/v) and sample solutions of varying concentrations (0.02, 0.025, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, and 0.12% w/v) were prepared with distilled water. A panel consisting of five trained subjects compared the sweetness of sample solutions to a sucrose solution of a given concentration. The mouth was rinsed with water twice before begining the analyses and 3 times between sample analyses. Sample size was usually 2–5 mL. The sweetener solution was held in the mouth for ~3 s and then spit out. The panelists scored the sweetness intensity of the sample solutions as less than, equal to, or greater than that the sucrose solution provided.

Threshold Measurement. A descending series at decreasing concentrations of sample solution was prepared. Sweetness intensity was approximately quantified by determining the dilution rate required to reach the threshold level for recognizing the sample as sweet.

RESULTS AND DISCUSSION

The synthetic route of a new kynurenine derivative, 3-(4-chloroanthraniloyl)-DL-alanine (6) is illustrated in Scheme I. The elementary analyses as well as spectroscopic and nuclear magnetic resonance data supported the structure.

Result of relative sweetness evaluation is shown in Table I. The sweetness intensity of newly synthesized compound 6 was affected by the concentration of the sucrose reference. The compound 6 was 80 times sweeter than sucrose at the levels of sweetness intensity of 2 and 4% sucrose

Table I. Sweetness of 4-Chloro-DL-kynurenine Relative to That of Sucrose

sucrose ref, %	isosweet concn of 4-chloro-DL-kynurenine, %[a]
2	0.025
4	0.05
6	0.08
8	0.11

[a] Concentration at which all judges scored the sweetness of the 4-chloro-DL-kynurenine solution as equal to or greater than that the sucrose solution provided.

Table II. Taste Thresholds for Sucrose and 4-Chloro-DL-kynurenine

stimulus	threshold[a] % by wt
sucrose	0.8
4-chloro-DL-kynurenine	0.01

[a] Concentration judged sweet by all panelists.

references, while its relative sweetness was somewhat dropped at levels above 6% sucrose reference levels. It was noteworthy that **6** did not differ significantly from sucrose in aftertaste and off flavor.

The result of threshold measurement is shown in Table II. The threshold concentration of **6** for sweetness was $^1/_{80}$ of that of sucrose on a weight base. This indicated that **6** was ~80 times as sweet as sucrose.

From the above two sensory analyses, the relative sweetness of **6** is ~80 times sweeter than sucrose. Since **6** is in the racemic form, it is possible that, as with tryptophan, only one optically active isomer may be sweet.

Although the compound **6** has not been tested for safety for use in foods, our data seems useful for structural modification of aromatic amino acids in order to design a new class of intensely sweet compounds.

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Cleanup of Methanolic Extract in High-Pressure Liquid Chromatography of Fructose, Glucose, and Sucrose in Onion Powder

A minicolumn of aluminum oxide could be used to clean up onion extract in 80% buffered methanol. Without this step, the injector and analytical column of the high-pressure liquid chromatograph (LC) became seriously damaged after many injections. Buffered methanol, pH 6.6, prepared with acetate buffer instead of phosphate was suitable for extraction and injection of soluble sugars from onion powder. Phosphate salts in methanol form needles that are dangerous for the analytical column of the high-pressure LC.

After publication of the paper of Gorin (1979), our institute purchased a Waters analytical high-pressure liquid chromatograph (LC) and found that the type of onion extract described in that paper could not be directly injected without damage to the injector and column. Samples had to first be clarified. Furthermore, the use of methanol with phosphate buffer, as in that paper, was dangerous as phosphate salts in methanol tended to crystallize after 1–2 days as needles, whatever the temperature (5 or 20 °C). So we looked for a means of cleaning up the sample and for another buffer instead of phosphate. Gutman (1974) found aqueous acetate satisfactory, even at 100 °C, although theoretically its pK seems inappropriate for buffering in the pH range 6.0–6.6. At lower pH, sucrose is hydrolyzed.

MATERIALS AND METHODS

Onion powder was that prepared Feb. 14, 1977, from bulbs not treated with maleic hydrazide (Gorin, 1979). The subsample was never removed nor opened during storage under nitrogen at −70 to −80 °C.

Buffered methanol was prepared as described before (Gorin, 1979) except for replacement of phosphate buffer by 0.1 M acetate buffer, pH 6.6 (Gutman, 1974), of which 20 mL was mixed with 80 mL of absolute methanol (Merck, 6009).

Reference Solutions of Soluble Sugars. Fructose (Merck, 5323), glucose (Merck, 8342 E), and sucrose (Merck, 7651 E) were separately dissolved in buffered methanol (100 mL), so that the volume of 10 μL injected into the high-pressure LC contained 25, 50, 75, or 100 μg of the sugar.

Preparation of the Minicolumn. This column was based on the work of Dunmire and Otto (1979). Aluminum oxide (Merck, 1097) was activated at 105 °C for 90 min and poured into a Pasteur pipet (Figure 1).

Onion Extract. Of the subsample, 500 mg (in former tests 100 mg) was suspended in buffered methanol (10 mL) at 55 °C and, after 15 min, centrifuged at 35280g for 30 min at 5 °C. The supernatant constituted the extract. Despite the increase in the ratio of powder to buffer, soluble sugars were still completely extracted from the

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