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D-(3-Carboxy-4-hydroxyphenyl)-glycine and Related Amino Acids in Higher Plants

ANDERS KJÆR and P. OLESEN LARSEN

Department of Organic Chemistry, Royal Veterinary and Agricultural College, Copenhagen, Denmark

Paperchromatographic analysis of an unhydrolyzed seed extract of *Reseda luteola* L. reveals its content of a new, aromatic plant amino acid, isolated and identified as (3-carboxy-4-hydroxyphenyl)-glycine on comparison with synthetic material, prepared by a new synthetic route.

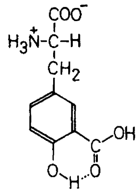
The synthetic racemate is resolved into enantiomers by fractional crystallization of the diastereoisomeric salts with L-arginine. The absolute configuration of the enantiomers is deduced upon comparison of rotation values with those of close analogues with known absolute configuration.

Isolation from seed material by ion exchange technique under conditions minimizing racemization yields D-*(3-carboxy-4-hydroxyphenyl)-glycine*, accompanied by the racemate. This is considered to be the first unambiguously established D-amino acid isolated from higher plants.

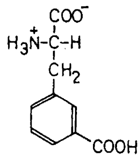
From the same seed extract DL-(3-carboxyphenyl)-glycine, L-(3-carboxyphenyl)-alanine, and L-(3-carboxy-4-hydroxyphenyl)-alanine, all previously encountered in higher plants, are isolated in pure form. The implications of this remarkable co-occurrence of four *m*-carboxy-substituted amino acids are briefly discussed.

During studies in this laboratory of free amino acids in plants containing isothiocyanate-producing glucosides, L-(3-carboxy-4-hydroxyphenyl)-alanine (*m*-carboxy-L-tyrosine) (I) was recently isolated from seed of *Reseda odorata* L. (Resedaceae).[1] The same amino acid, as well as L-3-(3-carboxyphenyl)-alanine (*m*-carboxy-L-phenylalanine) (II), was subsequently obtained from a seed extract of the crucifer *Lunaria annua* L.;[2] inferred from paper chromatography, this species also contained (3-carboxyphenyl)-glycine (III).[2] The last two amino acids have formerly been identified by Thompson *et al.*[3,4] as constituents of bulbs of *Iris tingitana.**

* No rotation value was reported for the *Iris* isolate of (3-carboxyphenyl)-glycine;[3] according to a private communication from Dr. J. F. Thompson, however, the specimen possessed a small, negative rotation ($[\alpha]_D -6°$) in aqueous solution.



I



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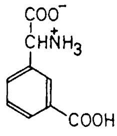

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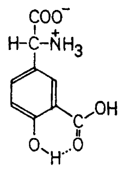


IV

Paper chromatography of the amino acid fraction in unhydrolyzed seed extracts of a number of species of the family Resedaceae revealed the contents of an amino acid possessing R_F-values slightly lower than those of (I), but having properties such as a strong, blue fluorescence in UV-light, a characteristic fluorescence activation spectrum, and a purple reaction with ferric chloride in common with this amino acid.[1] A conspicuous difference was noticed, however, in the behaviour towards ninhydrin; with this reagent, (I) reacted normally whereas the unknown amino acid produced a yellow spot, changing within a few days through various shades of brown to a normal purple colour. Similar colour changes have been described for benzylamine and certain derivatives, including phenylglycine; *p*-substitution with hydroxy- or methoxy-

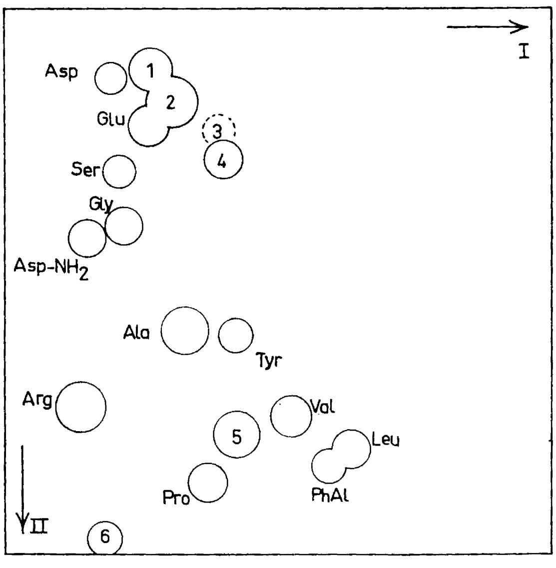
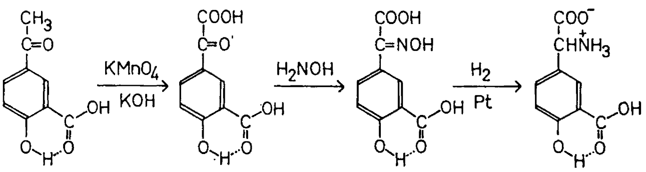


Fig. 1. Two-dimensional paper chromatogram of the amino acid fraction in seeds of *Reseda luteola* L. Solvent I: butanol:acetic acid:water (12:3:5), solvent II: phenol:water: conc.ammonia (120:30:1); treated with 0.2 % ninhydrin in acetone. The spot marked by a dotted line was detectable only in concentrated fractions.
1: D-(3-Carboxy-4-hydroxyphenyl)-glycine. 2: L-3-(3-Carboxy-4-hydroxyphenyl)-alanine. 3: (3-Carboxyphenyl)-glycine. 4: L-3-(3-Carboxyphenyl)-alanine. 5: γ-Aminobutyric acid. 6: Unknown neutral or basic compound.

groupings is known to retard the colour change.[5] The initial, yellow ninhydrin spot of (3-carboxyphenyl)-glycine is reported to undergo a similar change at room temperature.[6] The combined evidence at this stage suggested that the unknown amino acid was a (3-carboxy-4-hydroxyphenyl)-glycine (IV), strongly supported by its paperchromatographic identity with a synthetic, racemic specimen of the latter, prepared according to literature directions.[7]

Seed material of *Reseda luteola* L. (dyer's weed, weld) served as a convenient source for the isolation of the unknown amino acid.* On two-dimensional chromatograms of unhydrolyzed seed extracts of this species, the new amino acid appeared together with (I), (II), several common amino acids, and an unknown constituent, which is being further studied (Fig. 1). The first attempt at isolation, in which an extract prepared from 750 g of seed was processed by standard ion exchange techniques, ultimately yielded 4 mg of a preparation which proved identical with authentic DL-(3-carboxy-4-hydroxyphenyl)-glycine [7] with regard to spectroscopical and chemical properties. The naturally derived material possessed no, or, at most, only a very low, optical activity, indicating complete or extensive racemization, when the notorious, high rotation of optically active phenylglycine and its derivatives is considered (*cf.* Table 1).** Since loss of optical activity during isolation was throughout conceivable in view of the rather facile racemization of phenylglycine,[9,10] a synthesis of the pure enantiomers was undertaken in order to assess the extent of racemization under conditions simulating those prevailing during the experimental operations.

The reported synthesis of DL-(3-carboxy-4-hydroxyphenyl)-glycine [7] did not proceed satisfactorily in our hands; hence, an alternative synthetic route was devised according to the following scheme:



V VI VII VIII

Oxidation of 5-acetylsalicylic acid (V), conveniently produced by Fries-rearrangement of acetylsalicyclic acid,[11] afforded a stable hydrate of the phenylglyoxylic acid (VI). This was converted into the oximino-acid (VII), which, in its turn, was subjected to hydrogenolysis to give the racemic amino acid (VIII). In accord with older statements,[7] the amino acid separated as a dihydrate; the hygroscopic, anhydrous preparation rapidly took up water to form a stable hemihydrate.

* The employed seed was commercially obtained from H. Bornträger, Offstein, Kreis Worms, Germany.

** For the same reason, the low rotation of the (3-carboxyphenyl)-glycine obtained from *Iris tingitana* suggests that this preparation also was essentially racemic (*cf.* Ref.[8]).

In view of the potential risk of racemization, a resolution method was sought not involving excessive treatment with strong acids or bases. Since enzymic methods had been without avail in the case of (3-carboxyphenyl)-glycine [7] they were considered unlikely to be more successful in the present case. Chemical resolutions of phenylglycine have formerly been achieved by means of optically active sulphonic acids.[12] In view of the strongly acid properties of the present amino acid, however, it appeared more reasonable to attempt resolution through salt formation with optically active bases. Since exploratory experiments with brucine, morphine, (*R*)-1-phenylethylamine, and (*S*)-amphetamine were not very promising, recourse was taken to the readily accessible, basic protein amino acids. Though not entirely new, the utilization of one amino acid as resolving agent for another seems to be limited to patent claims. Thus, L-glutamic acid forms crystalline salts with L-arginine [13] and L-lysine, and has been used to resolve racemic lysine;[14,15] conversely, L-lysine has served as a resolving base for glutamic and aspartic acid.[16] Both L-lysine and L-arginine produced crystalline salts with the present aromatic amino acid and partial resolution was achieved in exploratory experiments; as L-arginine seemed to be the more promising base, this amino acid was selected for further experiments. From the salt of lowest solubility, believed to be homogeneous or nearly so, levorotatory (3-carboxy-4-hydroxyphenyl)-glycine separated on acidification to its isoionic point (pH 2.4). From the mother liquors, containing the diastereoisomeric salt, the dextrorotatory isomeride was obtained. The enantiomers separated from aqueous solutions as monohydrates, conspicuously more soluble in water than the racemic modification. Incidentally, this difference in solubility, prevailing also in the case of (3-carboxyphenyl)-glycine,[8] created difficulties in separating optically active material from the partly racemic, natural products and is probably responsible for the isolation only of the racemic (3-carboxy-4-hydroxyphenyl)-glycine in

Table 1. Molecular rotations at the D-line of optically active, *m*-carboxy-substituted amino acids. When not otherwise stated, conditions are those presented in the experimental part.

Amino Acid	1 N HCl	Water	Phosphate buffer, pH 7, 0.2 M	1 N NaOH
D-(3-Carboxy-4-hydroxyphenyl)-glycine	−277°	−218°	−206°	−207°
(3-Carboxy-4-hydroxyphenyl)-glycine of natural derivation	−176°[a]		−130°	
D-(3-Carboxyphenyl)-glycine	−248°[b]	−173°[b]		
(3-Carboxyphenyl)-glycine of natural derivation		− 12°[f]		
L-Phenylglycine	+ 254°[c]	+ 172°[c]		+ 170°[d]
L-3-(3-Carboxy-4-hydroxyphenyl)-alanine	+ 2°[e]		− 67°	− 17°
L-3-(3-Carboxyphenyl)-alanine	+ 9°	− 34°	− 53°	− 2°

[a] Determined in 0.6 N HCl. [b] Ref.[8] [c] Ref.[17] [d] Determined in this laboratory (*c* 3.1). [e] Ref.[1] [f] According o Dr. J. F. Thompson (private communication).

the first approach described above.* The solid phase IR-spectra of the optically active monohydrate, the racemic dihydrate, and the racemic hemihydrate of (3-carboxy-4-hydroxyphenyl)-glycine exhibited small, but characteristic differences.

In Table 1, the molecular rotations of the active amino acid at various pH-values are presented, together with the corresponding data for L-phenylglycine [17] and D-(3-carboxyphenyl)-glycine.[8] For comparison, the rotations of L-3-(3-carboxy-4-hydroxyphenyl)-alanine and L-3-(3-carboxyphenyl)-alanine are likewise included. The absolute configuration of optically active phenylglycine has been established beyond doubt through several correlations.[18,19] Again, the configuration of the enantiomeric (3-carboxyphenyl)-glycines and some derivatives has been determined in this laboratory as described in the preceding paper.[8] As seen from Table 1, the levorotatory amino acids have higher negative rotations in hydrochloric acid than in water. This shift, interpreted in terms of the Clough-Lutz-Jirgenson rule,[20,21] as well as all other rotation values, indicate that the levorotatory enantiomer of the amino acid belongs to the D-series (IV).

Table 2. Stability data for D-(3-carboxy-4-hydroxyphenyl)-glycine.

Solvent	Room Temperature	Heating
1 N HCl	stable	48 h at 80°, 5 % racemization
1 N Formic acid	stable	48 h at 80°, 30 % racemization
Phosphate buffer pH 7, 0.2 M	stable	48 h at 100°, discolouration
1 N NH_3, aq	stable	24 h at 60° discolouration
1 N NaOH	discolouration after 7 days	

In order to examine the stability of the optically active amino acid, it was exposed to the conditions shown in Table 2. Clearly, hot acidic solutions induced slow racemization whereas heating in neutral or basic solutions resulted in decomposition. The rates of racemization and decomposition were sufficiently slow, however, to warrant a new attempt to isolate the amino acid, again from 750 g of *R. luteola* seed, but this time under conditions minimizing racemization. The procedure employed is outlined in Fig. 2 and involves extensive use of chromatography on basic ion exchange resins. The application of acetic and formic acid solutions could not be entirely avoided, but care was taken to conduct the operations at reasonably low temperatures.

* Most likely, that same explanation applies to the isolation of virtually racemic (3-carboxyphenyl)-glycine from *Iris tingitana*.[3,8]

Disintegrated and defatted seed

Extraction with 70 % methanol

Strongly acid ion exchange resin in the acid form

Ammonia eluate

Strongly basic ion exchange resin in the formate form (elution with formic acid)

- 1. Fraction (unbound neutral and basic amino acids)
- 2. Fraction — Strongly basic ion exchange resin in the acetate form (elution with acetic acid)
  - Glutamic acid
  - Chromatography on a strongly basic ion exchange resin in the acetate form (elution with acetic acid)
    - Aspartic acid
    - L-3-(3-Carboxyphenyl)-alanine (66 mg)
    - DL-(3-Carboxyphenyl)-glycine (6 mg)
- 3. Fraction
  - L-3-(3-Carboxy-4-hydroxy-phenyl)-alanine (160 mg)
  - Chromatography on a strongly basic ion exchange resin in the formate form (elution with formic acid)
    - L-3-(3-Carboxy-4-hydroxy-phenyl)-alanine (150 mg)
    - DL-(3-Carboxy-4-hydroxy-phenyl)-glycine (8 mg) / Partly racemic D-(3-carboxy-4-hydroxyphenyl)-glycine (4 mg)

Fig. 2. Fraction scheme for amino acids in seed extracts of *Reseda luteola* L.

Paperchromatographic analysis of concentrated fractions obtained during the first isolation, had indicated their contents of trace amounts of (3-carboxyphenyl)-glycine. Accordingly, a small quantity (6 mg) of this amino acid was isolated, in addition to (3-carboxy-4-hydroxyphenyl)-glycine. Besides these two phenylglycine derivatives, L-3-(3-carboxy-4-hydroxyphenyl)-alanine and L-3-(3-carboxyphenyl)-alanine were isolated in crystalline form. Consequently, all four, naturally occurring *meta*-carboxysubstituted amino acids have here been isolated from the same source.

The isolated sample of (3-carboxyphenyl)-glycine exhibited infra-red and ultraviolet absorption spectra indistinguishable from those of the synthetic, racemic modification. It was homogeneous, as estimated from paper chromatography, and its chemical identity was confirmed by co-chromatography with authentic material. However, the isolated amino acid was devoid of significant optical rotation when measured over the wave length range 300—600 mμ. In view of the small, negative rotation of the (3-carboxyphenyl)-glycine isolated from *Iris tingitana*, it seems likely that this belongs to the D-series (*cf*. Ref.[8]).

The isolated sample of (3-carboxy-4-hydroxyphenyl)-glycine was divided into two crops by fractional crystallization. Both appeared identical with a synthetic specimen on basis of paper chromatography and UV-spectra. The first crop (8 mg) represented nearly racemic material and gave an IR-spectrum indistinguishable from that of the authentic racemate; its optical rotation was very low and of negative sign. The second crop (4 mg) consisted of partly racemic D-(3-carboxy-4-hydroxyphenyl)-glycine (IV), as apparent from its IR-spectrum, determined in the solid state, as well as from its rotation value, presented in Table 1. Estimated from rotation data, this fraction contained about 65 % of optically active material. Calculated on basis of the total amount of (3-carboxy-4-hydroxyphenyl)-glycine isolated, the optical purity amounted to only about 20 %. The plain rotatory dispersion curve of the isolate was similar to that of synthetic D-(3-carboxy-4-hydroxyphenyl)-glycine (Fig. 3), but of much smaller ordinate values.

On basis of the above evidence it is concluded *that at least one of the two phenylglycine derivatives isolated from seed of* Reseda luteola *L. possesses the* D-*configuration.*

The partly or completely racemic nature of the isolated specimens, of course, raises the question as to the sterical homogeneity of the amino acids in the intact seed material. Lack of seed material precluded experiments, such as, *e.g.*, determination of the recovery percentage of pure enantiomers added to the seed, to test the extent of racemization taking place during isolation. Hence, it cannot be exluded that the seed, in fact, contains the pure D-forms of the two amino acids.

According to paperchromatographic analysis, twelve examined seed samples of species belonging to the family Resedaceae all contained one or more of the four *m*-carboxy-substituted amino acids. A few analyses of whole *Reseda* plants revealed the presence of the amino acids in the fresh parts. Formerly, three of the four amino acids has been identified in seeds of the crucifer *Lunaria annua* L. and the closely related *Lunaria rediviva* L.[2] Again, paperchromatographic data from this laboratory indicate the occurrence of 3-(3-carboxyphenyl)-alanine in the cruciferous species *Arabis hirsuta* Scop. and *Berteroa*

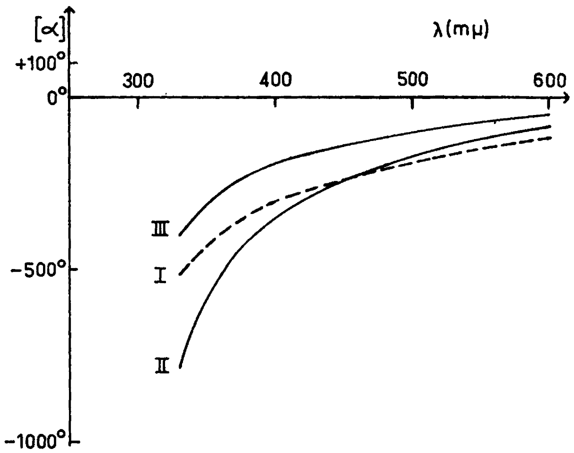


Fig. 3. Rotatory dispersion curves of D-(3-carboxyphenyl)-glycine (I), D-(3-carboxy-4-hydroxyphenyl)-glycine (II), and partly racemic, natural (3-carboxy-4-hydroxyphenyl)-glycine (III). Conditions are those outlined in the experimental part.

incana (L.) DC. Whereas the above distribution is comprehensible, in view of the generally accepted phylogenetic alliance of Cruciferae and Resedaceae, the reported occurrence of some of the same amino acids in a member of the monocotyledonous family Iridaceae suggests a much broader distribution within the plant kingdom.

The present demonstration of the occurrence of D-amino acids in higher plants is not entirely without precedent in the literature; however, most, or all, of the older claims, listed in a survey by Neuberger,[22] should be accepted only with considerable reservation, because of insufficient experimental documentation to exclude racemization or secondary reactions during isolation. The recent demonstration of the racemic nature of a specimen of 4-methyleneproline,[23] isolated from *Eriobotrya japonica*,[24] leaves undecided the possibility of racemization taking place during isolation. Hence, to our best knowledge, the above described isolation of a D-phenylglycine derivative represents the first unambiguous demonstration of the presence of D-amino acids in higher plants.*

Clearly, the deviating structure of phenylglycine and its derivatives, when compared with the ordinary amino acids, may render the established D-configuration less surprising. In this connection it may be of interest that several D-enantiomers of the common amino acids, unknown from higher plants, have been reported as products of metabolic reactions in microorganisms. The here reported D-configuration of the first phenylglycine derivatives in higher plants has its counterpart in the established L-configuration of phenyl-

* *Added in proof.* Since this paper was submitted for publication the occurrence of a malonyl conjugate of D-tryptophane in higher plants has been reported.[33]

glycine [25–27] and N-methylphenylglycine,[28] both obtained as degradation products of antibiotics of microbial origin. Phenylglycine, of unknown configuration, has formerly been isolated from a mutant strain of *Neurospora crassa* [29] and it has been demonstrated that the isolate, or the same amount of racemic phenylglycine, in certain microorganisms requiring phenylalanine and tyrosine for growth, can overcome a deficiency in these amino acids. Aromatic compounds with a substitution pattern similar to that of (3-carboxy-4-hydroxyphenyl)-glycine are rare in Nature; the closest analogue may be 3-acetyl-6-methoxybenzaldehyde, isolated some years ago from *Encelia farinosa* (Compositae).[30]

The biosynthesis of *m*-carboxy-substituted amino acids in higher plants is a subject of study in this laboratory at the present.

EXPERIMENTAL

Melting points were determined in capillary tubes in an Anschütz-Hershberg apparatus equipped with fully immersed thermometers. The standard rate of heating was 2° per min.

Where not otherwise stated, rotations were measured in a 1 dm tube. Determinations performed in an 0.5 dm tube are designated: "microdetermination".

Racemization and stability experiments were performed in closed ampoules in 0.8 % solutions.

Paper Chromatography. In accord with the strongly acid properties of the *m*-carboxy-substituted aromatic amino acids, R_F-values proved to be highly dependent on the pH-value of the applied solution when solvent systems of low buffering capacity towards acids (*e.g.* butanol:acetic acid:water) were employed.[1] The following R_F-values were determined for the four *m*-carboxy-substituted amino acids by descending chromatography on Whatman paper No. 1 at 25°:

	(1)[a]	(2)[b]	(3)[c]
(3-Carboxyphenyl)-glycine	0.23	0.39	0.44
(3-Carboxy-4-hydroxyphenyl)-glycine	0.11	0.27	0.34
3-(3-Carboxyphenyl)-alanine	0.28	0.40	0.50
3-(3-Carboxy-4-hydroxyphenyl)-alanine	0.18	0.31	0.48

[a] Phenol:water:conc.NH_3 (120:30:1) (w/v/v);
[b] Butanol:acetic acid:water (12:3:5). The amino acids applied in neutral solution;
[c] same, but applied in acid solution.

All isolated *m*-carboxy-substituted amino acids were homogeneous on paper chromatography; the identities were confirmed upon co-chromatography with authentic specimens.

The fluorescence activation spectra were measured on chromatograms developed in butanol:acetic acid:water. A Zeiss PMQ II spectrophotometer with attachment for the scanning of paper chromatograms, was used. A filter (Chance OY 10) was inserted between the chromatogram and the photoelectric cell. Maximal fluorescence was determined as a function of the wavelength of the incident light. For (3-carboxy-4-hydroxyphenyl)-glycine, maximum was found at 304 mμ, for 3-(3-carboxy-4-hydroxyphenyl)-alanine at 308 mμ.

Syntheses

5-Acetylsalicylic acid (V). A mixture of *O*-acetylsalicylic acid (76 g), nitrobenzene (633 ml) and aluminium chloride (114 g) was maintained at 60° for 4 h. After cooling, the solution was poured on to a mixture of conc. hydrochloric acid (100 ml), ice (1 kg), and water (2 l). The mixture was extracted with ether (10 l), and the organic phase was extracted with potassium hydroxide solution (30 g in 500 ml of water). On acidification, the crude 5-acetylsalicylic acid separated. After three recrystallizations from dilute ethanol, 5-acetylsalicylic acid (V) (45.6 g, 60 %), m.p. 213–218°, was obtained. Lit. values: 210°,[31] 216–217°.[32]

(3-Carboxy-4-hydroxyphenyl)-glyoxylic acid (VI). An aqueous solution (20 ml) of 5-acetylsalicylic acid (720 mg) and potassium hydroxide (550 mg) was vigorously agitated in a water bath at 40°. Within 1 1/2 h a solution of potassium permanganate (1300 mg) and potassium hydroxide (1040 mg) in water (100 ml) was added. After 18 h at room temperature, manganese dioxide was removed by filtration. The filtrate was adjusted to pH 5 with conc. hydrochloric acid and concentrated *in vacuo* to one tenth of the original volume. The solution was then adjusted to pH 1.5 with conc. hydrochloric acid and the precipitate, consisting of potassium chloride, 5-acetylsalicylic acid, and (3-carboxy-4-hydroxyphenyl)-glyoxylic acid, was removed by filtration. From this precipitate, (3-carboxy-4-hydroxyphenyl)-glyoxylic acid (66 mg) was isolated by extraction with methyl ethyl ketone. Unreacted 5-acetylsalicylic acid was removed as an insoluble residue upon treatment of the extracted material with water. From the original mother liquor more potassium chloride and ketoacid separated on addition of conc. hydrochloric acid. The keto acid was separated from the inorganic salt by extraction with methyl ethyl ketone and precipitated from this solvent with pentane. A sample (168 mg) with m.p. 185–188° (decomp.) was obtained. The total yield was 26 %. An analytical specimen was produced by recrystallizations from methyl ethyl ketone:chloroform:heptane. After air drying, a monohydrate was obtained, m.p. 189–191° (decomp.) (Found: C 47.39; H 3.65. Calc. for $C_9H_6O_6$, H_2O: C 47.38; H 3.53).

The permanganate oxidation was subsequently performed on a larger scale (140 g of 5-acetylsalicylic acid). By careful processing of all mother liquors a total yield of 31 % was obtained.

DL-*(3-Carboxy-4-hydroxyphenyl)-glycine (VIII).* A solution of the glyoxylic acid (VI) (24 g), hydroxylammonium sulfate (29 g), and sodium acetate (61 g) in water (700 ml) was kept at room temperature for a week, whereupon the solution was adjusted to pH 1 with conc. hydrochloric acid. The precipitated mixture of unchanged starting material and (3-carboxy-4-hydroxyphenyl)-oximinoacetic acid (VII) was dissolved in sodium hydroxide (80 ml, 2 N) and water (300 ml). Acetic acid (20 ml) was added, since the hydrogenation only proceeded at a reasonable rate in slightly acid solution, and the solution was hydrogenated for 8 h over Adam's platinum catalyst (1 g), during which time 3.5 l of hydrogen (21°, 760 mm Hg) were absorbed. The amino acid, partly separating during hydrogenation, was brought into solution by adjusting the pH-value to 5.5 with sodium hydroxide. After removal of the catalyst, the solution was applied to a strongly acid ion exchange resin (Amberlite IR 120, 4 × 50 cm) in the acid form. After washing the column with water, the amino acid was eluted with ammonia (3 l, 1 N). The eluate was evaporated to dryness, dissolved in water (150 ml), the solution adjusted to pH 2.4 with conc. hydrochloric acid, and the resulting precipitate of DL-(3-carboxy-4-hydroxyphenyl)-glycine, 2 H_2O (9 g, 35 % calculated on basis of the keto acid) filtered off. An analytical specimen was produced by recrystallization from water, m.p. above 250° (decomp.) (Found: C 43.70; H 5.45; N 5.68; H_2O (determined as weight loss at 50° and 0.01 mm over phosphorus pentoxide) 14.22. Calc for $C_9H_9O_5N$, 2 H_2O: C 43.73; H 5.30; N 5.67; H_2O 14.58). The ultraviolet absorption spectra were measured in a concentration of 0.09 μ equiv./ml. Because of association, extinctions below 240 mμ are highly dependent on concentration; λ_{max} and ε_{max}: (a) 0.5 N HCl: 303 mμ, 3440; 213 mμ, 25 600; (b) phosphate buffer pH 7, 0.025 M: 300 mμ, 3 460; 211 mμ, 25 000; (c) 0.5 N NaOH: 306 mμ, 3 330; 250 mμ, 8 100; 221 mμ, 13 000. When the water of crystallization was removed as described, the hygroscopic anhydrous amino acid remained. Exposed to the atmosphere, water was taken up to form the hemihydrate (Found: C 49.25; H 4.61; N 6.33; H_2O 3.75. Calc. for $C_9H_9O_5N$, 1/2 H_2O: C 49.10; H 4.58; N 6.36; H_2O 4.09).

Resolution

Resolution of synthetic (3-carboxy-4-hydroxyphenyl)-glycine. The racemic amino acid dihydrate (12.3 g) and L-arginine (8.62 g) were dissolved in hot water (300 ml). Ethanol (220 ml) was added to the hot solution, which was left overnight in the refrigerator. A crystalline salt was isolated and dried at 0.01 mm over phosphorus pentoxide (4.36 g), $[\alpha]_D^{21}$ $-41.7°$ (*c* 1.3, water). Recrystallization from aqueous ethanol yielded 3.42 g, $[\alpha]_D^{24}$ $-42.9°$ (*c* 1.2, water), an additional recrystallization 2.32 g, $[\alpha]_D^{26}$ $-43.3°$ (*c* 1.3, water). This preparation, believed to represent a homogeneous diastereoisomeride, was very hygroscopic and took up water to a sesquihydrate when exposed to the atmosphere (Found: C 44.08; H 6.45; N 16.68; H_2O 6.04. Calc. for $C_{15}H_{23}O_7N_5$, 1 1/2 H_2O: C 43.70; H 6.32; N 16.99; H_2O: 6.56).

The salt (670 mg of the sesquihydrate)was dissolved in water (25 ml) and the solution adjusted to pH 2.4 with hydrochloric acid (1.66 ml, 1 N). After a night in the refrigerator, a small precipitate (47 mg) of partly racemic D-(3-carboxy-4-hydroxyphenyl)-glycine, H_2O ($[\alpha]_D^{23}$ $-85.6°$, (*c* 0.86, phosphate buffer pH 7, 0.2 M)) was removed. The mother liquor yielded a second crop consisting of pure D-*(3-carboxy-4-hydroxyphenyl)-glycine, H_2O* (Found: C 47.27; H 4.97; N 6.09. Calc. for $C_9H_9O_5N$, H_2O: C 47.17; H 4.84; N 6.11), $[\alpha]_D^{24}$ $-90.1°$ (*c* 0.7, phosphate buffer pH 7, 0.2 M), $[\alpha]_D^{22}$ $-121°$ (*c* 0.75, 1 N HCl), $[\alpha]_D^{25}$ $-105°$ (*c* 0.7, 1 N formic acid), $[\alpha]_D^{25}$ $-90.3°$ (*c* 0.88, 1 N NH_3, aq), $[\alpha]_D^{25}$ $-90.3°$ (*c* 0.68, 1 N NaOH), $[\alpha]_D^{22}$ $-95°$ (*c* 0.71, water, supersaturated). The water of crystallization could not be removed at 50° and 0.01 mm over phosphorus pentoxide. The rotatory dispersion curve was determined in a 0.04 % solution in 1 N HCl as shown in Fig. 3.

To the first mother liquor, more ethanol was added. A crystalline precipitate (11.4 g after drying over phosphorus pentoxide, $[\alpha]_D^{23}$ $-2.1°$ (*c* 1.3 water)) was removed by filtration. The mother liquor was evaporated to dryness *in vacuo*, and the residue dissolved in water (100 ml). The solution was adjusted to pH 2.4 with hydrochloric acid (4.22 ml, 4 N). After cooling, a precipitate of partly racemic material was obtained (1.34 g, $[\alpha]_D^{25}$ $+ 15.1°$ (*c* 0.75, phosphate buffer pH 7, 0.2 M). The mother liquor was concentrated *in vacuo*. After cooling overnight in the refrigerator, a crystalline product, (2.76 g, $[\alpha]_D^{25}$ $+ 88.5°$ (*c* 0.73, phosphate buffer pH 7, 0.2 M)) was isolated by filtration. Recrystallization from water afforded an analytical specimen of L-*(3-carboxy-4-hydroxyphenyl)-glycine, H_2O* (Found: C 47.28; H 4.84; N 6.18), $[\alpha]_D^{24}$ $+ 85.6°$ (*c* 0.72, phosphate buffer pH 7, 0.2 M). The infra-red absorption spectrum was indistinguishable from that of the enantiomer.

Isolation

750 g of seed of *Reseda luteola* L. were disintegrated in a ball mill and refluxed with three 2.5 l portions of carbon tetrachloride. The air-dried residue was refluxed for 2 h with methanol:water (2.5 l, 70 %), cooled and filtered. The extraction was repeated three times with fresh portions of solvent. The combined filtrates were then evaporated *in vacuo* to a syrup, which was partly dissolved in water (0.5 l), filtered, and again evaporated to dryness (52 g). The residue was dissolved in water (0.5 l) and the total amino acid fraction was bound on a strongly acid ion exchange resin (Amberlite IR 120, 4 × 50 cm) in the acid form. After washing the column with water, the amino acids were eluted with ammonia (3 l, 1 N). The eluate was evaporated *in vacuo* to a dark syrup (5.6 g), dissolved in water (25 ml), and applied to a strongly basic ion exchange resin (Dowex 1 × 4, 20—50 mesh, 2.2 × 33 cm) in the formate form. The effluent was collected in fractions of 250 drops (*ca.* 20 ml). The column was washed first with water, and after fraction 16 eluted with 1 N formic acid. Fractions 3—14 contained the basic and neutral compounds (first fraction). Fractions 15—23 contained only glutamic acid and were discarded. Fractions 24—40 contained glutamic acid, aspartic acid, 3-(3-carboxyphenyl)-alanine, (3-carboxyphenyl)-glycine, and traces of 3-(3-carboxy-4-hydroxyphenyl)-alanine (second fraction). Fractions 41—90 contained 3-(3-carboxy-4-hydroxyphenyl)-alanine and (3-carboxy-4-hydroxyphenyl)-glycine (third fraction).

The second fraction was evaporated *in vacuo* to a semicrystalline solid (1.2 g), dissolved in water (40 ml) and applied to a strongly basic ion exchange resin (Dowex 1 × 4, 20—50 mesh, 2.2 × 29 cm) in the acetate form. The effluent was collected in fractions of 300 drops (*ca.* 24 ml). The column was washed with water and eluted with 1 N acetic acid after fraction 6. Fractions 24—65 contained aspartic acid, 3-(3-carboxyphenyl)-alanine, and (3-carboxyphenyl)-glycine. All the glutamic acid and most of the aspartic acid appeared in earlier fractions and were discarded. Fractions 24—65 were evaporated *in vacuo* to a crystalline residue (230 mg), dissolved in water (1 ml) and applied to a strongly basic ion exchange resin (Dowex 1 × 8, 200—400 mesh, 0.9 × 92 cm) in the acetate form. The effluent was collected in fractions of 150 drops (*ca.* 12 ml). The column was eluted with 0.5 N acetic acid. Aspartic acid appeared in fractions 19—23 which were discarded. 3-(3-Carboxyphenyl)-alanine appeared in fractions 35—41 and (3-carboxyphenyl)-glycine in fractions 51—57.

Fractions 35—41 were evaporated *in vacuo* to a crystalline residue (66 mg). Recrystallization from water afforded a pure sample of *L-3-(3-carboxyphenyl)-alanine*, $[\alpha]_D^{26}$ $-16.2°$ (*c* 0.96, water (supersaturated)), $[\alpha]_D^{27}$ $+4.1°$ (*c* 0.97, 1 N HCl), $[\alpha]_D^{22}$ $-25.4°$ (*c* 0.68, phosphate buffer pH 7, 0.2 M), $[\alpha]_D^{24}$ $-1.1°$ (*c* 0.48, 1 N NaOH). Lit. value $[\alpha]_D^{24}$ $-15.8°$ (*c* 0.95, water (supersaturated)).[2] The infra-red absorption spectrum was identical with that of an authentic sample.[2]

Fractions 51—57 were evaporated *in vacuo* to a semicrystalline residue, dissolved in water (1 ml), and freed from traces of metals by treatment with hydrogen sulfide and subsequent centrifugation. Further purification was accomplished by means of a strongly basic ion exchange resin (Dowex 1 × 8, 200—400 mesh, 0.6 × 3 cm). The amino acid was bound to the resin and eluted with 0.5 N acetic acid. Water and acetic acid was removed from the eluate (4.5 ml) by evaporation *in vacuo* to give crystalline *DL-(3-carboxyphenyl)-glycine* (6 mg). The infra-red absorption spectrum was identical with that of an authentic sample of DL-(3-carboxyphenyl)-glycine.[8] The ultraviolet absorption spectrum was determined in 0.5 N HCl: λ_{max}: 275 mμ, 228 mμ, and 205 mμ. Lit. value: λ_{max} 275 mμ in water.[3]

The third fraction was evaporated *in vacuo* to a crystalline residue (600 mg) and dissolved in water (30 ml). The solution was adjusted to pH 9 with ammonia (4 N), treated with hydrogen sulfide to remove traces of metals, filtered, and evaporated to dryness again. The residue was dissolved in water (7 ml), the solution was adjusted to pH 2.4 with hydrochloric acid (4 N) and cooled. The precipitated L-3-(3-carboxy-4-hydroxyphenyl)-alanine (160 mg) was isolated by filtration. Recrystallization from water afforded a pure specimen of *L-3-(3-carboxy-4-hydroxyphenyl)-alanine*, $[\alpha]_D^{26}$ $-7.8°$ (*c* 0.9, 1 N NaOH), $[\alpha]_D^{24}$ $-29.9°$ (*c* 0.63, phosphate buffer pH 7, 0.2 M). Lit. value $[\alpha]_D^{25}$ $-7.7°$ (*c* 0.9, 1 N NaOH).[1] The infra-red absorption spectrum was identical with that of an authentic sample.

The mother liquor from the precipitated L-3-(3-carboxy-4-hydroxyphenyl)-alanine was applied to a strongly basic ion exchange resin (Dowex 1 × 8, 200—400 mesh, 0.9 × 86 cm) in the formate form. The effluent was collected in fractions of 150 drops (*ca.* 12 ml). The column was eluted with 1 N formic acid, 3-(3-carboxy-4-hydroxyphenyl)-alanine appeared in fractions 34—40, and (3-carboxy-4-hydroxyphenyl)-glycine appeared in fractions 48—56.

Fractions 34—40 were evaporated *in vacuo* to yield an additional crop of crystalline L-3-(3-carboxy-4-hydroxyphenyl)-alanine (150 mg).

Fractions 48—56 were evaporated *in vacuo* to a semicrystalline solid and dissolved in hot water (4.5 ml). After cooling, the precipitated *DL-(3-carboxy-4-hydroxyphenyl)-glycine*, $2H_2O$ (8 mg) was isolated by centrifugation. The isolated material exhibited an infra-red absorption spectrum indistinguishable from that of synthetic material. The ultraviolet absorption spectrum was measured in 0.5 N HCl and was identical with that of synthetic material. The optical activity of the material was very low, $[\alpha]_D^{23}$ $-4.2°$ (*c* 0.5, 1 N HCl).

The mother liquor from the nearly racemic amino acid was treated with charcoal to remove coloured impurities, centrifuged, and evaporated to dryness. The residue was dissolved in water, treated with hydrogen sulfide to remove traces of metals and centrifuged. Further purification was again accomplished by using a strongly basic ion exchange resin (Dowex 1 × 8, 200—400 mesh, 0.5 × 2.4 cm) in the formate form. The amino acid was bound to the resin and eluted with 1 N formic acid. Water and formic acid were removed from the eluate (6 ml) by evaporation *in vacuo* to give the crystalline, *partly racemic D-(3-carboxy-4-hydroxyphenyl)-glycine* (4 mg). The isolated material exhibited an infra-red spectrum suggestive of a mixture of optically active and racemic material. The ultraviolet absorption spectrum was measured in 0.5 N HCl and was identical with that of synthetic material. The following rotations were determined: $[\alpha]_D^{27}$ $-77°$ (*c* 0.7, 0.6 N HCl, microdetermination), $[\alpha]_D^{21}$ $-57°$ (*c* 0.5, phosphate buffer pH 7, 0.2 M, microdetermination). The rotatory dispersion cruve was determined in a 0.035 % solution in 1 N HCl and is shown in Fig. 3.

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