Pyrogenesis of Succinic Acid and Dimethylmaleic Anhydride from Aspartic Acid

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Aspartic acid has been pyrolyzed under a slow stream of nitrogen at temperatures ranging from 350 to 650 °C. Succinic acid was the major nonvolatile acid product observed, with maximum yield (12%) at 650 °C. Aspartic acid-*4*-^{13}C at 500 °C gave succinic acid with unchanged ^{13}C enrichment. Fumaric acid and maleic acid are possible intermediates in this transformation. Fumaric acid, mixed with alanine and pyrolyzed, was converted in part to succinic acid. A plausible reaction path for the formation of succinic acid in this work is proposed, and the possible relevance of the present work to certain other pyrolysis reactions is discussed. A minor product of the pyrolysis of aspartic acid at 400 °C and higher temperatures was dimethylmaleic anhydride; under the same conditions alanine gave negligible amounts of dimethylmaleic anhydride. Pyrolysis of mixtures of alanine and aspartic acid gave a sevenfold or better improvement in the yield of dimethylmaleic anhydride, and the yield of this product was essentially unchanged when aspartic acid was replaced by fumaric acid in the mixed pyrolysis. Carbon-13 tracer experiments were used to establish the stoichiometry of the mixed pyrolysis reaction leading to dimethylmaleic anhydride. These experiments showed also that the carboxyl carbon atoms of aspartic acid become equivalent at some stage of the reaction. Evidence is presented for a close relationship between the formation of dimethylmaleic anhydride from alanine and aspartic acid or fumaric acid and the reduction of fumaric acid to succinic acid by alanine at elevated temperatures. A reaction scheme is presented to account for the ^{13}C distribution in the products.

Succinic acid has been observed as a product of pyrolysis of ovalbumin at reduced pressure,[1] and as a product of pyrolysis of malic acid and of sodium lactate, using nitrogen as a carrier gas.[2] Further, succinic acid has been observed as a constituent of cigarette smoke[3] and cigar smoke[4] in quantities suggesting that it originates, in part, from pyrolysis reactions. As will be seen from the work reported here, likely precursors of the pyrolysis-generated succinic acid are malic acid and aspartic acid. We have found that succinic acid is the major nonvolatile acid product observed in the pyrolysis of aspartic acid.[5]

The diversity of starting materials leading to succinic acid in pyrolysis reactions suggests the possibility that succinic acid can be formed by recombination of $\cdot CH_2CO_2H$ fragments, following carbon–carbon bond dissociation at elevated temperature in appropriate starting materials. We have carried out some pyrolysis experiments with ^{13}C-labeled aspartic acid that were designed to show to what extent succinic acid may be formed by such reaction from aspartic acid.

Dimethylmaleic anhydride was noted as a minor product in the conversion of aspartic acid to succinic acid and also in the course of using our controlled pyrolysis apparatus[6] to study pyrolysis of soy protein and an amino acid mixture having the same amino acid composition. These findings stirred our interest because the material pyrolyzed contained no carbon skeletons corresponding to that of dimethylmaleic anhydride and also because of the possible relevance of this finding to food preparation at high temperature and to tobacco smoking. Further, the pyrogenesis of dimethylmaleic anhydride from aspartic acid, and from alanine mixed with aspartic acid or fumaric acid, might provide evidence on the mode of formation of succinic acid in these same reactions and in certain other reactions leading to succinic acid, such as the pyrolysis of malic acid,[7] the dry distillation of ovalbumin,[8] and in tobacco smoking.[9,10]

At 350 °C, DL-aspartic acid was converted mainly into brown, amorphous polyaspartic acid.[11] Pyrolysis with extensive decomposition and carbonization became pronounced by 400 °C in our apparatus, and at 650 °C, the carbonlike residue amounted to 25% of the weight of the starting material Succinic acid was the major nonvolatile acid product, observed as its dimethyl ester in gas–liquid chromatography (GLC). The yield of succinic acid increased with increasing temperature of pyrolysis up to our highest temperature, 650 °C (Table I). Fumaric acid and maleic acid were observed as very minor products in this work. Our methyl esterification procedure for GLC estimation of nonvolatile acids was inappropriate for oxalic acid and other acids whose esters are easily hydrolyzed, but dimethyl oxalate was observed in our product mixtures, usually in trace amount. The four identified dimethyl esters accounted for 97 ± 1% of the total area under product elution peaks.

Pyrolysis of aspartic acid-*4*^{13}C, 13.4 ± 1.0% ^{13}C enrichment, at 500 °C gave succinic acid-^{13}C with ^{13}C enrichment unchanged within experimental error (Table II). To the extent that succinic acid was produced by recombination of $\cdot CH_2{}^{13}CO_2H$ fragments, ^{13}C enrichment would be increased and some succinic acid-*1,4*-$^{13}C_2$ would be formed. The results of measurements summarized in Table II show, to the contrary, that such a reaction path was negligible. Nor did fumaric acid or maleic acid from aspartic acid-*4*-^{13}C show any significant difference in ^{13}C enrichment from that of the parent compound. Pyrolysis at 500 °C of a mixture of alanine and aspartic acid-*4*-^{13}C (60/40 mol/mol) also gave succinic acid-^{13}C and fumaric acid-^{13}C without significant changes in ^{13}C enrichment from that of aspartic acid.

Some experiments were designed to test the possibility that fumaric acid and/or maleic acid were involved as intermediates in the conversion of aspartic acid to succinic acid. A mixture of aspartic acid and fumaric acid (86/14 mol/mol) was pyrolyzed at 500 °C. The yield of succinic acid from this reaction was 24% greater than that obtained from aspartic acid alone under the same conditions (Table III). In another experiment alanine containing 21 mol % fumaric acid on pyrolysis at 600 °C gave a 7% yield of succinic acid (based on starting fumaric acid), substantially better than that from a mixture of alanine and aspartic acid under the same conditions (Table IV). Since alanine alone gave no succinic acid or fumaric acid on pyrolysis, it can be seen from these experiments that fumaric acid can be reduced to succinic acid by an α-amino acid at elevated temperatures. Pyrolysis at 600 °C of a mixture of alanine and maleic anhydride gave a mixture of nonvolatile acids qualitatively similar to that from pyrolysis of aspartic acid, as judged by GLC examination of their trimethylsilyl esters.

In a control experiment, pyrolysis of fumaric acid at 600 °C gave fumaric acid (40% recovery as dimethyl ester) and maleic acid (46% yield as dimethyl ester).

Table I. Pyrolysis of Aspartic Acid

Temp, °C	Yields of dicarboxylic acid products, mmol[a]			
	Succinic	Fumaric	Maleic	Total
350	0.7[b]	0.3[b]	0.04[b]	1.0[b]
400	3.6	0.4	0.06	4.0
500	5.5	1.1	0.09	6.7
600	5.7	0.9	0.09	6.7
600	5.6[c]	0.1[c]	0.02[c]	5.7
650	6.2	0.4	0.05	6.7

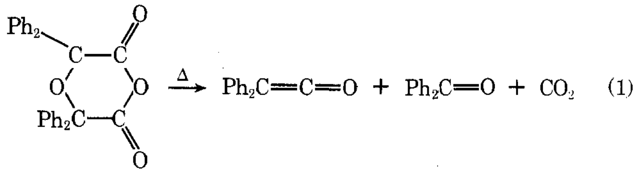
[a] Yields, mmol per 100 mmol of starting aspartic acid, were estimated by gas–liquid chromatography of dimethyl ester mixtures using an internal standard and comparing with known mixtures. [b] Based on unrecovered starting material. [c] Amorphous polyaspartic acid, obtained from the experiment at 350 °C, was employed in this experiment.

Table II. Mass Spectrometry Results for Aspartic-*4*-^{13}*C* Acid and for Succinic, Fumaric, and Maleic Acid Products from Pyrolysis at 500 °C, Examined as Dimethyl Esters

M/z	Assignment	Excess ^{13}C,[a] %
M+ (not observed)	$CH_3O_2C^*CH_2CH_2CO_2CH_3^+$	
115, 116	$CH_3O_2C^*CH_2CH_2CO^+$	12.5 ± 0.8[b]
87, 88	$CH_3O_2C^*CH_2CH_2^+$	7.2 ± 0.8
M+	$CH_3O_2C^*CH{=}CHCO_2CH_3^+$ (*trans*)	14.0 ± 0.3[b]
85, 86	$CH_3O_2C^*CH{=}CH^+$	6.5 ± 0.6
M+ (not observed)	$CH_3O_2C^*CH{=}CHCO_2CH_3^+$ (*cis*)	
113, 114	$CH_3O_2C^*CH{=}CHCO^+$	13.3 ± 0.3
85, 86	$CH_3O_2C^*CH{=}CH^+$	6.5 ± 0.6
M+ (not observed)	$CH_3O_2C^*CH_2CHNH_2CO_2CH_3^+$	
102, 103	$CH_3O_2C^*CH_2CH{=}NH_2^+$	14.7 ± 0.8
88, 89	$CH_3O_2CCH{=}NH_2^+$	1.1 ± 0.2

[a] Percentage ^{13}C together with standard deviation of five or more measurements, corrected for natural ^{13}C abundance and mass spectrometer background by comparison with corresponding unenriched materials. [b] Results for the product from pyrolysis of aspartic-*4*-^{13}*C* acid alone were indistinguishable from those obtained for the corresponding product from alanine mixed with aspartic-*4*-^{13}*C* acid, within experimental uncertainty. Both results are included here.

At elevated temperatures benzilic acid undergoes decomposition to diphenylacetic acid, benzophenone, and carbon dioxide.[12] Since carbon monoxide was not produced in this reaction, the benzophenone product could not have been formed by the familiar decarbonylation route. At somewhat lower temperatures, benzilic acid underwent dehydration to the cyclic ether anhydride 1,[13,14] and when 1 was heated above 250 °C, it decomposed into carbon dioxide, benzophenone, and diphenylketene[13] (eq 1).



(1)

1

The dehydration of benzilic acid to 1 followed by decomposition of 1 accounts very well for the overall decomposition of benzilic acid at elevated temperatures, since water produced in the first step can react with diphenylketene to give the observed diphenylacetic acid.

Mandelic acid undergoes decarbonylation to produce benzaldehyde and carbon monoxide.[15] Hurd[16,17] has shown that thermal decomposition of mandelic acid also produces phenylacetic acid and carbon dioxide. Further, Hurd and Raterink prepared an ether anhydride from mandelic acid, analogous to 1, and showed that it decomposed at 250 °C in the manner of 1, in part.[17]

Table III. Pyrolysis of Aspartic Acid Mixtures at 500 °C

Second component	Yields of dicarboxylic acids, mmol[a]		
	Succinic	Fumaric	Maleic
None	5.5	1.1	0.09
Fumaric acid, 14 mol %	6.8	2.8[b]	0.00
Alanine, 60 mol %	5.4	0.2	0.00

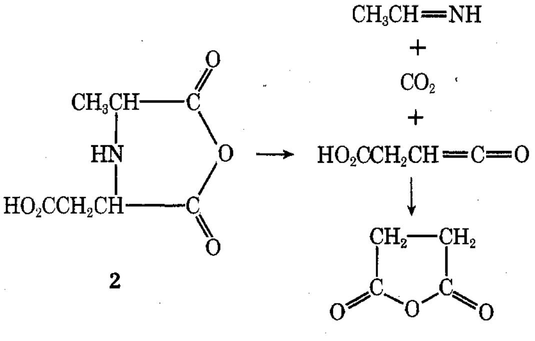
[a] See corresponding footnote, Table I. [b] Percentage recovery.

Table IV. Pyrolysis of Alanine Mixtures at 600 °C

Second component	Yields of dicarboxylic acids, mmol[a]		
	Succinic	Fumaric	Maleic
None	0.0	0.0	0.0
Aspartic acid, 40 mol %	4.7	0.1	0.0
Fumaric acid, 21 mol %	7.3[b]	1.7[c]	0.0
Succinic acid, 20 mol %	88[c]	0.0	0.0

[a] See corresponding footnote, Table I. [b] mmol per 100 mmol of unrecovered fumaric acid. [c] Percentage recovery.

The α-hydroxy acid to ether anhydride to ketene pathway established for the formation of diphenylacetic and phenylacetic acids (above) can be applied to the formation of succinic acid from malic acid by pyrolysis: reversible dehydration of malic acid to fumaric acid occurs at elevated temperature,[18] and the required cyclic ether anhydride intermediate is a plausible product resulting from addition of malic acid to the carbon–carbon double bond of fumaric acid (or maleic acid) followed by dehydration. An analogous pathway seems reasonable for the formation of succinic acid from aspartic acid (partial deamination of aspartic acid to fumaric acid followed by addition of aspartic acid to the fumaric acid produced) and from alanine plus fumaric acid. In the latter case, the hypothesized cyclic intermediate 2 is unsymmetrical and alternative modes of decomposition are possible (eq 2 and 3).



(2)

2

$$2 \longrightarrow CH_3CH{=}C{=}O + CO_2 + HO_2CCH_2CH{=}NH \quad (3)$$

In the present work the yield of succinic acid from amorphous polyaspartic acid was almost as good as that from aspartic acid itself under the same conditions (Table I). Consequently, it seems reasonable to extend the proposed reaction path, with slight modification, to account for the formation of fatty acids and succinic acid in the pyrolysis of ovalbumin.[1]

On the basis of the proposed reaction path, the overall re-

Table V. Dimethylmaleic Anhydride from Aspartic Acid by Pyrolysis

Temp, °C	Yield,[a,b] %
400	0.06
500	0.10
600	0.09
600	0.05[c]
650	0.06

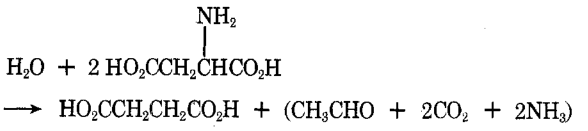
[a] Yields were estimated by means of gas–liquid chromatography, using an internal standard and comparison with known mixtures. [b] Calculated on the basis of the reaction, 2 aspartic acid → dimethylmaleic anhydride + $2NH_3$ + H_2O + $2CO_2$. [c] Amorphous polyaspartic acid was employed in this experiment.

Table VI. Dimethylmaleic Anhydride from Mixtures Containing Alanine

Temp, °C	Second component	Yield,[a] %
600	None	<0.04[b]
500	Aspartic acid (40 mol %)	0.67[c]
600	Aspartic acid (40 mol %)	0.67[c]
600	Fumaric acid (21 mol %)	0.63[d]

[a] See corresponding footnote of Table I. [b] This upper limit was calculated on the basis of the supposed reaction, 2 alanine → dimethylmaleic anhydride + $2NH_3$ + H_2O. [c] Basis: aspartic acid + alanine → dimethylmaleic anhydride. [d] Basis: fumaric acid + alanine → dimethylmaleic anhydride.

action leading to succinic acid from aspartic acid by pyrolysis is, after hydrolysis, as shown in eq 4.

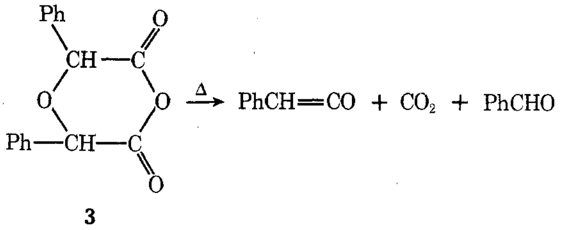


$$H_2O + 2\,HO_2CCH_2\overset{NH_2}{\overset{|}{C}}HCO_2H \longrightarrow HO_2CCH_2CH_2CO_2H + (CH_3CHO + 2CO_2 + 2NH_3) \quad (4)$$

Assuming the stoichiometry of eq 4, the yield of succinic acid from aspartic acid at 650 °C was 12.4%.

The yields of dimethylmaleic anhydride obtained at several temperatures from aspartic acid are given in Table V. Pyrolysis of alanine alone gave a negligible yield of dimethylmaleic anhydride but alanine exerted a remarkable effect on the yield of dimethylmaleic anhydride from aspartic acid (Table VI). Further, we observed that the yield of dimethylmaleic anhydride obtained from the pyrolysis of alanine mixed with fumaric acid was comparable to that obtained from the pyrolysis of alanine–aspartic acid mixture. There is a striking parallel between the yields of dimethylmaleic anhydride and yields of succinic acid for most of these reactions,[19] and we suspected a close relationship between the reaction paths leading to succinic acid and dimethylmaleic anhydride, involving one or more common intermediates.

A possible clue to the mode of formation of both succinic acid and dimethylmaleic anhydride in our pyrolysis reactions is furnished by results obtained by Hurd and Raterink[17] in the thermal decomposition of the cyclic ether anhydride **3**. The weight of evidence indicated strongly that most of the products formed from **3** arose from the two reactions, eq 5 and 6.[16,17]



(5)

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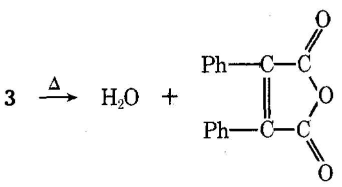
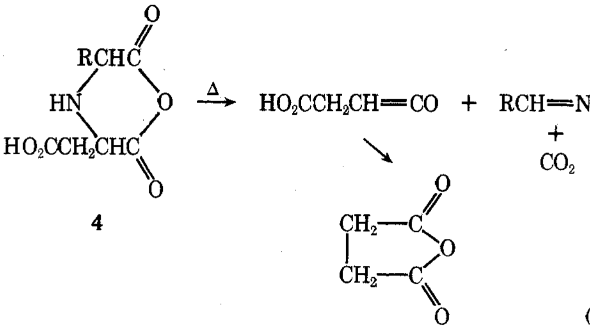


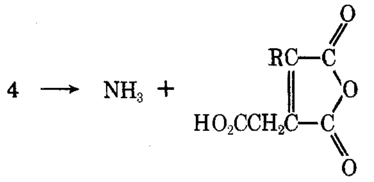
(6)

In the pyrolysis reactions reported herein, succinic acid and dimethylmaleic anhydride were produced together, or neither was produced (pyrolysis of alanine alone). In the reactions leading to both products, fumaric acid was either present in the mixture pyrolyzed or it could be produced from aspartic acid by loss of ammonia (Tables V and VI). In these reactions a cyclic imino anhydride (**4**) analogous to **3** could be produced as a transitory intermediate by addition of α-amino acid to the carbon–carbon double bond of fumaric acid (or, possibly, maleic acid). Thermal decomposition of **4** could produce both succinic anhydride, eq 7, and disubstituted maleic anhydride, eq 8.



(7)

a, R = HO_2CCH_2
b, R = CH_3



(8)

5

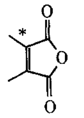
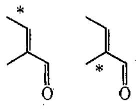
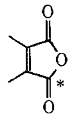
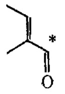
a, R = HO_2CCCH_2
b, R = CH_3

Because **5** is a β,γ-unsaturated acid it would be expected to undergo decarboxylation readily and give rise to relatively volatile dimethylmaleic anhydride.

Added alanine increases the yield of dimethylmaleic anhydride from aspartic acid (Tables V and VI) but does not increase the yield of succinic acid from aspartic acid. This effect of added alanine on the yield of dimethylmaleic anhydride from aspartic acid suggests that the rate of reaction 8 relative to that of reaction 7 is more favorable for **4b** than for **4a**. Accordingly, our interpretation of the mode of formation of dimethylmaleic anhydride leads to the expectation that most of the dimethylmaleic anhydride produced in the pyrolysis of alanine–aspartic acid mixtures arises from the addition of alanine to fumaric acid and/or maleic acid derived from aspartic acid, followed by dehydration to **4b** and thermal decomposition of **4b** to **5b**.

Carbon-13 tracer experiments were designed to test the major points of the above interpretation of the formation of dimethylmaleic anhydride from alanine-aspartic acid mixtures: the correct reaction stoichiometry can be established; and it can be determined whether or not the carboxyl carbon atoms of aspartic acid actually become equivalent at some stage of reaction prior to decarboxylation.

Table VII. Carbon-13 Labeled Dimethylmaleic Anhydride from Labeled Mixtures of Amino Acids at 500 °C: (a) Alanine-*3*-^{13}C and Unenriched Aspartic Acid, 60/40 mol %; (b) Aspartic-*4*-^{13}C Acid and Unenriched Alanine, 40/60 mol %

	Labeled component of mixture	Dimethylmaleic anhydride	
		M/z 126, 127	*M/z* 82, 83
	$C^*H_3CHNH_2CO_2H$		
Excess $^{13}C^a$	18.6 ± 0.8%[b]	19.2 ± 0.4%	18.0 ± 0.3%
	$HO_2C^*CH_2CHNH_2CO_2H$		
Excess $^{13}C^a$	13.4 ± 1.0%	6.7 ± 0.7%	3.2 ± 0.6%

[a] Percentage ^{13}C together with the standard deviation of five or more measurements, corrected for natural ^{13}C abundance and mass spectrometer background by comparison with the corresponding unenriched material. [b] Measurements made on the methyl *N*-acetylester. [c] Measurements made on dimethyl esters of the starting amino acid and derived succinic, fumaric, and maleic acids.

Mass spectrometry results for two ^{13}C tracer experiments on the formation of dimethylmaleic anhydride from mixtures of alanine and aspartic acid are summarized in Table VII. Alanine-*3*-^{13}C mixed with unlabeled aspartic acid and pyrolyzed at 500 °C produced dimethylmaleic anhydride having the same excess ^{13}C as the starting alanine (reaction a, Table VII). This result shows that dimethylmaleic anhydride was produced exclusively from one molecule of alanine and one molecule of aspartic acid.

Aspartic-*4*-^{13}C acid was mixed with unlabeled alanine and pyrolyzed at 500 °C. The dimethylmaleic anhydride obtained from this reaction contained one-half the excess ^{13}C of the starting aspartic acid (Table VII, reaction b). This result confirmed the reaction stoichiometry found in the first tracer experiment, above, and also showed that the two carboxyl carbon atoms of aspartic acid do become equivalent at some stage of the reaction leading to dimethylmaleic anhydride, in agreement with the belief that fumaric acid and/or maleic acid is an intermediate.

It has been shown above that fumaric acid can be reduced to succinic acid by alanine at elevated temperatures, and fumaric acid and/or maleic acid has been implicated as an intermediate in the formation of succinic acid from aspartic acid by pyrolysis. The apparent close relationship, indicated by the present results, between succinic acid formation and formation of substituted maleic anhydride suggests that at least small amounts of substituted maleic anhydrides may be formed in certain other high-temperature reactions that have been reported to produce succinic acid.[10]

Experimental Section

Materials. DL-Aspartic acid, a commercial sample of good quality by ^1H NMR analysis, was further purified by recrystalliztion from water. DL-Alanine was similarly analyzed and purified. Maleic acid was obtained from maleic anhydride by recrystallization from water. Commercial 99+% fumaric acid was used without further purification. Dimethyl esters used for comparison purposes were prepared by standard methods and purified by distillation, except dimethyl oxalate, which was a commercial sample.

DL-Aspartic-*4*-^{13}C acid was obtained from Merck Sharp and Dohme Canada Ltd. and was diluted by recrystallization from water with added unlabeled aspartic acid.

L-Alanine-*3*-^{13}C was obtained from Merck Sharp and Dohme Canada Ltd. and was diluted with unlabeled DL-alanine. An aqueous solution of the diluted alanine-*3*-^{13}C, containing excess L isomer, was evaporated to dryness; the residue was ground in a mortar and stored over anhydrous calcium chloride.

Methyl DL-2-Acetaminopropionate. DL-Alanine (1.0 g, 11 mmol) was esterified, using absolute methanol and excess hydrogen chloride. Most of the solvent was removed by azeotropic distillation with benzene. The last of the solvent was removed at reduced pressure in a rotary evaporator, then the residue was dissolved in dilute sodium carbonate solution and treated with excess acetic anhydride, added in small portions with vigorous shaking. Water and acetic acid were removed at reduced pressure with a rotary evaporator and the residue was extracted with several small portions of chloroform. Chloroform was replaced by petroleum ether and the product was crystallized and recrystallized from ether–petroleum ether with cooling, and stored in a vacuum desiccator over anhydrous calcium chloride. The hygroscopic product melted at 40–43 °C, placed on the hot stage at 30 °C and heated rapidly, 0.71 g (45%): mass spectrum *m/e* 145, M^+; 114, $(M - 31)^+$; 86, $(M - 59)^+$. The ^1H NMR spectrum in Me_2SO-d_6 was appropriate for the expected structure.

δ, ppm	No. of protons	Assignment
1.3 doublet	3	CH_3CH
1.8 singlet	3	CH_3CO
3.6 singlet	3	CH_3O
4.4 quartet	1	CH_3CH

The L isomer of methyl 2-acetaminopropionate is reported to be an oil.[20]

Methyl 2-Acetaminopropionate-*3*-^{13}C. Carbon-13 labeled acetylalanine methyl ester, containing excess L isomer, was prepared from alanine-*3*-^{13}C as described for the corresponding unlabeled material, above. The chloroform extract of product was evaporated to give a slightly discolored oil which crystallized at room temperature in a vacuum desiccator over anhydrous calcium chloride.

Apparatus. The pyrolysis equipment used in this work has been described elsewhere.[6] The material to be pyrolyzed (2 g, powdered) was distributed as evenly as possible over a length of 100 cm in an 11-mm o.d. quartz tube. The tube was mounted on a rack, slightly declined from the horizontal. A small, ice-cooled trap was attached to the pyrolysis tube at the lower end, and a stream of nitrogen (60 ml/min) was passed through the tube to flush air and to serve as a carrier. Pyrolysis was accomplished by means of a mobile furnace which was motor driven along the rack at a rate of 1.35 cm/min.

Pyrolysis. The pyrolysis of aspartic acid and of alanine–aspartic acid mixtures at 400 °C and higher was accompanied by extensive carbonization and evolution of gases. Pyrolyzate preceded the furnace, being reheated by the furnace, and gradually moving down the pyrolysis tube. Very little condensate, mostly water droplets, collected in the cold trap. Pyrolyzate was washed from the tube with dilute aqueous sodium hydroxide, a little methanol, again with dilute alkali and, finally, with water.

Analysis. Methanol and most of the water were distilled from the trap and pyrolysis tube washings, above, and the concentrated alkaline solution was heated under reflux for a period of 24–48 h. This extended alkaline hydrolysis is designed to convert succinic acid derivatives to succinate for subsequent conversion to methyl succinate. Distillation was resumed and continued (with addition of water to the pot, if necessary) until the distillate was colorless, odorless, and neutral (discarded). The contents of the pot were acidified with hydrochloric acid and filtered and the acidic solution was submitted to continuous ether extraction (5–7 days). The residue obtained by evaporation of ether from the extract was dried by azeotropic distillation of water with benzene, then esterified with absolute methanol (50 ml) and concentrated sulfuric acid (1 ml). Most of the methanol was removed from the reaction mixture by codistillation with benzene and the mixture was neutralized with cold aqueous sodium carbonate solution. The aqueous layer was saturated with salt and extracted repeatedly with benzene. The combined benzene extracts were washed with water, dried, and concentrated by distillation of most of the solvent. In GLC examination[21] of product mixtures from aspartic acid, the esters of oxalic, fumaric, succinic, and maleic acids accounted for 97 ± 1% of the area under product elution peaks. A measured amount of dimethyl citraconate was added as an internal standard to ester mixtures for quantitative estimation of products.

Dimethyl esters of oxalic, fumaric, and succinic acids accounted for 70 ± 1% of the area under elution peaks in GLC of product mixtures from alanine–aspartic acid pyrolysis; several unidentified minor and trace products from alanine accounted for the remaining area. Dimethyl fumarate was used as an internal standard for quantitative estimation of dimethyl succinate. Results of GLC examination of the product mixture obtained from pyrolysis of alanine–fumaric acid were similar to those described for alanine–aspartic acid mixtures.

The identities of dimethyl esters of nonvolatile acid products were confirmed by isolation in preparative GLC[21] and comparison of their mass spectra with those of authentic samples and, except for dimethyl maleate, by means of infrared spectra and melting points.

In actual-scale control experiments, mixtures of succinic and fumaric acids were carried through the analytical procedure described above. The amounts of succinic and fumaric acids observed as dimethyl esters in GLC were 84 ± 2 and 82 ± 9% and the yield data for succinic and fumaric acids given in Tables I, III, and IV have been corrected accordingly. In a similar control experiment, not to scale, only moderate loss of maleic acid occurred during the analytical procedure; yields of this trace product were corrected using the somewhat arbitrary correction factor of 1.2.

Dimethylmaleic anhydride was removed from the hydrolyzed pyrolyzate by distillation with steam prior to continuous ether extraction of the nonvolatile acid products of pyrolysis. Gas–liquid chromatography of the organic material extracted from the steam distillate gave several trace peaks and one major elution peak, which corresponded in retention time to that of a purified sample of dimethylmaleic anhydride obtained from Aldrich Chemical Co. Dimethylmaleic anhydride was isolated by means of preparative GLC and its identity was confirmed by comparison of its mass spectrum, infrared spectrum, and melting point with those of authentic material.

Carbon-13 assays were done by the Mass Spectroscopy Center of the University of Kentucky.

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Registry No.—DL-Aspartic acid, 617-45-8; succinic acid, 110-15-6; dimethylmaleic anhydride, 766-39-2; methyl DL-2-acetamidopropionate, 26629-33-4; DL-alanine, 302-72-7.

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Study of Benzhydrylamine-Type Polymers. Synthesis and Use of *p*-Methoxybenzhydrylamine Resin in the Solid-Phase Preparation of Peptides[1]

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Starting with the phenylketopolystyrene–1% divinylbenzene intermediate, two routes have been compared for the synthesis of phenylaminomethylpolystyrene–1% divinylbenzene (benzhydrylamine-type polymer), useful for the preparation of C-terminal amide peptides by solid-phase synthesis. The desired primary amine containing polymer can best be obtained via the Leuckart reaction, while reduction of the oxime intermediate with metal hydrides leads to a large percentage of secondary amine. The Demjanov reaction has been adapted for the analysis of primary and secondary amine content of the polymers. Anisylaminomethylpolystryrene–1% divinylbenzene has also been synthesized by the Leuckart reaction, characterized with respect to primary and secondary amine content, and its usefulness illustrated by the synthesis of the hypothalamic hormone, Thyroliberin (TRH), and a series of model peptides.

In 1970 Pietta and Marshall[2] introduced into solid-phase peptide synthesis[3] a resin based upon a phenylaminomethylpolystyrene–2% divinylbenzene structure (benzhydrylamine resin), which attaches the growing peptide chain via a C-terminal amide bond to the polymeric support. The advantage over the utilization of a benzyl ester type linkage[4] for the