is solvent or hydrogen peroxide and k_6 is the rate constant for the reaction between quinone (T) and X. In the case of chlorogenic acid k_6 is probably small, since the quinone formed is more stabilized by resonance than *o*-benzoquinone. In addition, one of the two reactive positions (the 4,5-carbons) is substituted and is unavailable. This substitution must offer some steric hindrance to further reaction at carbon atom 5.

The autoxidation of chlorogenic acid also differs from that found for catechol by the occurrence of a measurable autocatalytic period up to *p*H 7.9. Joslyn and Branch[11] found no autocatalytic period for catechol.

The inverse hydrogen ion relationship shows that the catechol ion with one hydroxyl group ionized is very probably the reactive species. At *p*H values greater than 8.74 the rate constants increase as Joslyn and Branch[11] also found for catechol at high *p*H values. Their explanation that this is due to the oxidation of the ion with both hydroxyl groups ionized may also be true here. However, it is also possible that the high rate constants at the higher *p*H values may be due to an inaccuracy in the extrapolation of rate to zero time.

In some runs, which were carried up to 30% reaction, the oxygen uptake curve (*e.g.*, B of Fig. 1) appeared to be linear throughout. This linearity would seem to indicate a zero-order relationship for chlorogenic acid during the reaction, which might be expected if the products absorbed oxygen at a comparable rate to chlorogenic acid.

In order to determine what the course of the oxygen uptake would be during the reaction if the products did not absorb oxygen, equation 1 was integrated. Integration of equation 1 gives the equation

$$\text{Vol. } O_2 = 22400C_0[1 - \exp(-kP_{O_2}/(H^+)22400)] \quad (4)$$

where C_0 is the initial concentration of chlorogenic acid and C is the concentration of chlorogenic acid at any time during the reaction. The assumptions were made that (a) there is a 1:1 stoichiometry between oxygen and chlorogenic acid, (b) *p*H and P_{O_2} remain constant during the reaction, and (c) chlorogenic acid disappears by the first order rate law

$$C = C_0 \exp.(-k\,P_{O_2}t/(H^+)22400)$$

Using an average value of k for experiments 1–17 and the initial conditions of curve B (Fig. 1), curve D (Fig. 1) was calculated from equation 2.

As curve B does not differ significantly from curve D, it is not possible to draw any conclusions concerning the order of chlorogenic acid during the reaction or whether or not the products of the chlorogenic acid oxidation themselves absorb oxygen. Further research is being undertaken on this point.

Since the k we found is a product of k_1, k_4 and K, the energy of activation contains contributions from three sources and is therefore difficult to compare with the corresponding value found by Joslyn and Branch for catechol, which also includes contributions from similar terms.

Acknowledgment.—We wish to thank Dr. Benjamin Makower for many stimulating discussions during this work.

ALBANY 6, CALIFORNIA RECEIVED MAY 8, 1951

[CONTRIBUTION FROM THE CHEMOTHERAPY DIVISION, STAMFORD RESEARCH LABORATORIES, AMERICAN CYANAMID COMPANY]

Preparation of Peptides Using Mixed Carboxylic Acid Anhydrides

BY JAMES R. VAUGHAN, JR., AND RUTH L. OSATO

Mixed anhydrides of N-substituted aminoacids with α- or β-branched chain, low molecular weight aliphatic acids are formed under anhydrous conditions at low temperature and react with aminoacid esters to give good yields of the corresponding peptide esters. Anhydrides with isovaleric acid have been found particularly advantageous and have been used to prepare a series of peptides. The theoretical background for the work is discussed.

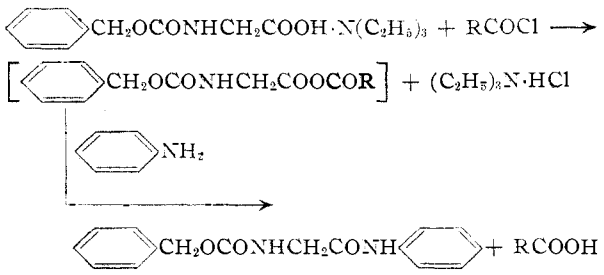
The use of mixed carboxylic acid anhydrides for the synthesis of peptides was reported recently by Wieland and Sehring,[1] who found that anhydrides of benzoic or acetic acid with N-substituted aminoacids react readily with aqueous solutions of a salt or an ester of a second aminoacid to give the corresponding peptide derivatives. No studies of the peptide reaction under anhydrous conditions were reported. This was of interest in view of the recent work of Emery and Gold[2] on the reactivities of mixed anhydrides in which they stress not only the importance of steric hindrance and charge-distribution in the molecular anhydride, but also the importance of the polarity of the solvent on the acylation ratio obtained when the anhydride reacts with an amine. According to these authors, under anhydrous conditions nucleophilic attack in the molecular anhydride will occur at that carbonyl carbon atom which has the lowest electron density and, other things being equal, which is less hindered sterically. Under aqueous conditions, however, these considerations become invalid, possibly due to ionization of the anhydride, and a marked change or even a reversal of the acylation ratio is observed. Predictions concerning the reaction in polar solvents, therefore, are uncertain. Under anhydrous conditions, however, the anhydride forming acid, the acylation reaction of which is to be suppressed, should be one in which the combined operation of an electronic and a steric effect would lessen the probability of nucleophilic attack at this part of the anhydride molecule and favor substitution at the

(1) T. Wieland and R. Sehring, *Ann.*, **519**, 122 (1950).

(2) A. R. Emery and V. Gold, *J. Chem. Soc.*, 1443, 1447, 1455 (1950).

relatively less hindered and relatively more electrophilic aminoacid carbonyl carbon.

In the present work, mixed anhydrides were formed between carbobenzoxyglycine and a series of both aliphatic and aromatic acids by reaction the respective acid chlorides with the triethylamine salt of carbobenzoxyglycine under anhydrous, standardized conditions. The anhydrides were then treated with aniline and the yields of carbobenzoxyglycinanilide taken as a measure of the acylation ratio. The over-all reaction may be formulated as



and the results are summarized in Table I.

TABLE I

PREPARATION OF CARBOBENZOXYGLYCINANILIDE BY THE MIXED CARBOXYLIC ACID ANHYDRIDE PROCEDURE

Acid chloride	Yield, %	M.p., °C. (cor.)[a]
Acetyl	36	144-145
Dimethylacetyl	65	146-147
Diethylacetyl	85	146-147
Trimethylacetyl	72	144-145
Isovaleryl	83	146-147
Isocaproyl	36	143-145
Heptanoyl	31[b]	139-141
Lauroyl	49	143-144
Hexahydrobenzoyl	37	146-147
Phenylacetyl	40	142-144
Hexahydrophenylacetyl	35	144-146
Trichloroacetyl	48	143-144
Benzoyl	62	141-142
Furoyl	46[b]	139-141
2-Methoxybenzoyl	18	143-144
4-Methoxybenzoyl	59[b]	<140
2,4-Dimethoxybenzoyl	38	146-147
3,4,5-Trimethoxybenzoyl	15[b]	139-141
2-Methylbenzoyl	38	140-142
2-Chlorobenzoyl	34	143-145
4-Chlorobenzoyl	63[b]	139-143
2,4-Dichlorobenzoyl	49[b]	<140
3,4-Dichlorobenzoyl	62[b]	<140
3-Bromobenzoyl	48[b]	<140
2-Iodobenzoyl	45	141-143

[a] The literature m.p. is 144° (ref. 1). [b] These products were still impure after 2-3 recrystallizations from methanol-water.

In agreement with the theory proposed, the α- and β-branched chain aliphatic acids, in which both the existing steric effects and the positive inductive effects of the alkyl groups reinforce each other in the desired manner, were found to be most satisfactory in increasing the acylation ratio of the mixed anhydride in favor of the aminoacid portion of the molecule. The aromatic acids, on the other hand, in which there exists an opposite electronic and inductive effect, gave less favorable results and a less pure product. Attempts to demonstrate an effect attributable to increased steric hindrance in these compounds by introducing various substituents into the benzene ring gave inconclusive results. When trimethylacetic, diethylacetic and isovaleric carbobenzoxyglycine anhydrides were further compared under anhydrous conditions in the preparation of ethyl carbobenzoxyglycyl-DL-phenylalaninate, the yield of peptide ester was 81, 84 and 86%, respectively. Under conditions using wet solvents, however, the yields of peptide ester were reduced to 58, 67 and 55%.

Since the isovaleric mixed anhydride (R = $(CH_3)_2CHCH_2$–) seemed eminently satisfactory for the purpose intended, isovaleryl chloride was chosen as a suitable reagent and used in the preparation of the peptide esters listed in Table II. As additional examples were obtained using the reaction, it was found to be more satisfactory than originally expected. The conditions of low temperature under which the reaction is carried out prevent excessive decomposition or by-product formation and lead to products of high initial purity. At higher temperatures the yields are lower and the products are less pure. Also, racemization does not seem to occur under these conditions, since several optically-active peptides prepared by this and by other methods have identical rotations. The expected difficulty of separating the desired peptide product from the product obtained when the isovaleric half of the anhydride molecule enters into an acylation reaction has not occurred. This side reaction doubtlessly contributes to the lowered yields of peptides encountered in some cases, but has not caused serious difficulty in the purification of the products obtained. Purification was effected by recrystallization from ethyl acetate petroleum ether or alcohol-water mixtures.

Experimental[3]

Carbobenzoxyglycinanilide.—A solution of 5.23 g. (0.025 mole) of carbobenzoxyglycine and 2.55 g. (0.025 mole) of triethylamine in 50 cc. of toluene was cooled to 0° and 3.01 g. (0.025 mole) of isovaleryl chloride added. After 2 hours at this temperature, during which time triethylamine hydrochloride separated, 2.34 g. (0.025 mole) of aniline was added. The reaction mixture was then stored at 8° overnight. The carbobenzoxyglycinanilide crystallized from the reaction mixture and was filtered off together with triethylamine hydrochloride, washed with water, dilute sodium hydroxide solution and dilute hydrochloric acid, and dried; wt. 5.87 g. (83% yield); m.p. 146-147°. These same reaction conditions were followed exactly using the acid chlorides listed in Table I. With the higher molecular weight aliphatic acid chlorides, and with most of the aromatic acid chlorides, the product separated initially in an impure state and was then recrystallized from aqueous methanol until the melting point was above 140°.

Preparation of Peptide Esters.—In general, the reaction conditions were essentially the same as those used in the preparation of carbobenzoxyglycinanilide. Two examples are given to illustrate minor variations. All solvents and reagents were carefully dried and purified, and precautions were taken to exclude moisture from the reaction mixtures.

Ethyl Carbobenzoxyglycyl-DL-phenylalaninate.—A solution of 0.025 mole of carbobenzoxyglycyl-isovaleric acid anhydride in 50 cc. of toluene was prepared as described above and 4.80 g. (0.025 mole) of ethyl DL-phenylalaninate was added. After storing overnight at 8°, the reaction mixture

(3) All melting points are corrected.

TABLE II

PREPARATION OF N-SUBSTITUTED PEPTIDE ESTERS

	Yield, %	M.p., °C. (cor.)	$[\alpha]^{24}D$ (ethanol)	Formula	Analyses,[g] % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Ethyl carbobenzoxyglycyl-DL-phenylalaninate[a]	86	91–92	.....							
Ethyl carbobenzoxyglycyl-L-tyrosinate[b]	77	125–127	+18.4 (c = 5)							
Ethyl carbobenzoxy-L-leucylglycinate[c]	70	103–104	−25.6 (c = 5)							
Ethyl dicarbobenzoxy-L-lysylglycinate[d]	58	89–91	−12.0 (c = 5)							
Methyl carbobenzoxy-L-leucyl-L-leucinate[e]	29	97–98.5	−35.3 (c = 10)							
Ethyl carbobenzoxy-L-leucyl-L-tyrosinate	52	115–117	−15.2 (c = 5)	$C_{25}H_{32}N_2O_6$	65.77	7.07	6.14	65.68	7.22	6.28
Ethyl carbobenzoxy-L-leucyl-DL-phenylalaninate	38	84–87	.....	$C_{25}H_{32}N_2O_5$	68.16	7.16	6.36	[h]	7.25	6.24
Ethyl carbobenzoxy-DL-alanyl-DL-phenylalaninate	48	114–116	.....	$C_{22}H_{26}N_2O_5$	66.31	6.58	7.03	66.22	6.65	7.17
Ethyl carbobenzoxyglycyl-DL-phenylalanyl-glycinate[f]	86	132–133	.....	$C_{23}H_{27}N_3O_6$	62.57	6.17	9.52	62.32	6.24	9.64
Ethyl phthalylglycyl-DL-phenylalaninate	68	149–150	.....	$C_{21}H_{20}N_2O_5$	66.30	5.30	7.37	66.28	5.36	7.43
Ethyl phthalylglycyl-L-tyrosinate	60	163–164	+43.0 (c = 2)	$C_{21}H_{20}N_2O_6$	63.63	5.05	7.07	63.40	5.10	7.27
Ethyl phthalylglycyl-L-leucinate	62	139–140	−24.5 (c = 2)	$C_{18}H_{22}N_2O_5$	62.41	6.40	8.09	62.20	6.44	8.05
Ethyl phthalyl-DL-alanyl-DL-valinate	40	110–130	.....	$C_{18}H_{22}N_2O_5$	62.41	6.40	8.09	62.34	6.56	8.26

[a] H. Neurath, *et al.*, *J. Biol. Chem.*, **170**, 221 (1947), give m.p. 90–91° (cor.). [b] M. Bergmann and J. S. Fruton, *ibid.*, **118**, 405 (1937), give m.p. 118°. [c] M. Bergmann, *et al.*, *ibid.*, 111, 225 (1935), give m.p. 103–104°; M. A. Nyman and R. M. Herbst, *J. Org. Chem.*, 15 108 (1950), give m.p. 99° and $[\alpha]^{25}D$ −26.8° (c = 2.6 ethanol). [d] M. Bergmann, *et al.*, *Z. physiol. Chem.*, **224**, 26 (1934), give m.p. 90°. [e] M. A. Nyman and R. M. Herbst, *ibid.*, give m.p. 97–98°. [f] Prepared from carbobenzoxyglycyl-DL-phenylalanine and ethyl glycinate. [g] We are indebted to Dr. J. A. Kuck and his staff of these laboratories for the microanalyses. The values reported are the average of two values differing by not more than 0.30. [h] A satisfactory carbon value was not obtained on this compound.

was washed with water and the product which had crystallized out was filtered off and washed with 3% sodium bicarbonate solution; wt. 5.29 g. (55% yield), m.p. 91–92°. The toluene layer was separated from the filtrate, washed rapidly with 3% sodium bicarbonate solution and diluted until cloudy with petroleum ether and cooled to crystallize a second crop of material; wt. 3.14 g. (33% yield); m.p. 90–91°. The two crops were combined and recrystallized from aqueous ethanol to give 8.25 g. (86%) of product melting at 91–92°.

Ethyl Carbobenzoxy-L-leucylglycinate.—A solution of 5.30 g. (0.02 mole) of carbobenzoxy-L-leucine and 2.04 g. (0.02 mole) of triethylamine in a mixture of 25 cc. of toluene and 25 cc. of chloroform was cooled to −5° and 2.41 g. (0.02 mole) of isovaleryl chloride added. After 1.5 hours a second, precooled solution of 2.79 g. (0.02 mole) of ethyl glycinate hydrochloride and 2.04 g. (0.02 mole) of triethylamine in 50 cc. of chloroform was added, and the reaction mixture was stored overnight at 8°. The organic solution was washed with water and with 3% sodium bicarbonate solution and diluted until cloudy with petroleum ether. On cooling, the product separated as colorless crystals; wt. 2.17 g. (31%), m.p. 103–104°. Concentration of the mother liquor almost to dryness in a stream of air and rediluting with petroleum ether gave a second crop of crystalline product; wt. 2.98 g. (43%); m.p. 102–103°. The two crops were combined and recrystallized from aqueous ethanol to give 4.90 g. (70%) of pure product melting at 105–106°.

STAMFORD, CONNECTICUT RECEIVED MAY 31, 1951

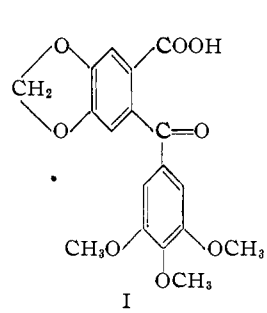
[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF BOSTON UNIVERSITY]

Synthesis of 2-(3′,4′,5′-Trimethoxybenzoyl)-piperonylic Acid[1,2]

BY WALTER J. GENSLER AND CARLOS M. SAMOUR

A synthesis for 2-(3′,4′,5′-trimethoxybenzoyl)-piperonylic acid is described according to the following sequence: homopiperonylamine, N-(trimethoxybenzoyl)-homopiperonylamine, 1-(3′,4′,5′-trimethoxyphenyl)-6,7-methylenedioxy-3,4-dihydroisoquinoline, 2-(3′,4′,5′-trimethoxybenzoyl)-4,5-methylenedioxystyrene and 2-(3′,4′,5′-trimethoxybenzoyl)-piperonylic acid. The over-all yield for the four steps is 60%.

In view of the possibility of elaborating picropodophyllin (II) or the related podophyllotoxin[3] from 2-(3′,4′,5′-trimethoxybenzoyl)-piperonylic acid (I), a convenient source of this keto acid would be desirable. Although 2-(3′,4′,5′-trimethoxybenzoyl)-piperonylic acid (I) had been obtained before, not only from picropodophyllin (II)[4] itself, but also from a synthetic dihydronaphthalene derivative[5] and from several derivatives of 1-(3′,4′,5′-trimethoxyphenyl)-6,7-methylenedioxyisoquinoline,[6] none of the paths constituted an attractive preparative method. We wish now to report on a practical synthesis of keto-acid (I).[7]



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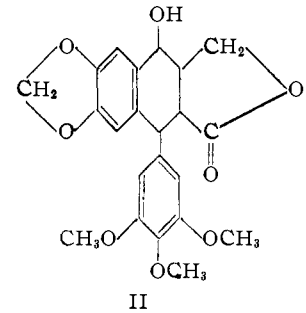

II

(1) This work was supported by American Cancer Society Grant-in-Aid No. CBC-6 as recommended by the Committee on Growth of the National Research Council.

(2) A summary of the material in this paper was presented in Boston, Mass., on April 3, 1951, before the Division of Organic Chemistry of the American Chemical Society.

(3) Podophyllotoxin, a tumor-damaging substance, is currently of interest as a potentially useful anti-cancer agent. For example, see the report by J. Leiter, V. Downing, J. L. Hartwell and M. J. Shear, *J. Nat. Cancer Inst.*, **10**, 1273 (1950).

(4) E. Späth, F. Wessely and E. Nadler, *Ber.*, **66**, 125 (1933).

(5) R. D. Haworth and T. Richardson, *J. Chem. Soc.*, 348 (1936). See also R. D. Haworth and J. R. Atkinson, *ibid.*, 797 (1938).

(6) W. Reeve and W. M. Eareckson, III, THIS JOURNAL, **72**, 5195 (1950).

(7) A brief account of this work has appeared, *ibid.*, **72**, 3318 (1950). In the preliminary report, reference to the work of Haworth and Richardson (ref. 5) was inadvertently omitted.