Experimental

The circular dichroism (C.D.) measurements were conducted using a Shimazu C.D. spectrophotometer "Dichrograph." The optical rotatory dispersion curves were determined by means of a Rudolph automatically recording spectropolarimeter. We are indebted to Dr. K. Kuriyama of the research laboratory, Shionogi and Co., Ltd., for the optical rotatory dispersion measurements.

Virosecurinine.—O.R.D. (*c* 0.02, methanol): $[\phi]_{589}$ +2360°, $[\phi]_{375}$ +20,200° (peak), $[\phi]_{265}$ −39,000°; C.D. (*c* 0.01, methanol): $[\theta]_{360}$[14] 0, $[\theta]_{320}$ +39,000, $[\theta]_{270}$ 0; for ultraviolet data, see Table I.

Allosecurinine.—O.R.D. (*c* 0.29, methanol): $[\phi]_{589}$ −2200°, $[\phi]_{345}$ −20,500° (trough), $[\phi]_{272}$ +58,400°; C.D. (*c* 0.01, methanol) $[\theta]_{350}$ 0, $[\theta]_{305}$ −46,000, $[\theta]_{270}$ 0; for ultraviolet data, see Table I.

Virosecurinine Methiodide.—O.R.D. (*c* 0.10, methanol): $[\phi]_{589}$ +4850°, $[\phi]_{400}$ +7200°, $[\phi]_{300}$ +53,800°, $[\phi]_{280}$ +251,000°.

Dihydrosecurinine.—O.R.D. (*c* 0.11, methanol): $[\phi]_{589}$ +19°, $[\phi]_{325}$ +528° (peak), $[\phi]_{273}$ −2170°; C.D. (*c* 0.15, methanol): $[\theta]_{330}$ 0, $[\theta]_{300}$ −2400, $[\theta]_{270}$ 0; λ_{max}^{MeOH} 215 mμ (log ε 4.21) and 300 mμ (log ε 2.12).

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The Tritiation of Diethyl Benzylacetamidomalonate by Exposure to Tritium Gas

HELEN KRIZEK,[1] ANTHONY J. VERBISCAR, AND WELDON G. BROWN

Argonne Cancer Research Hospital,[2] Chicago, Illinois

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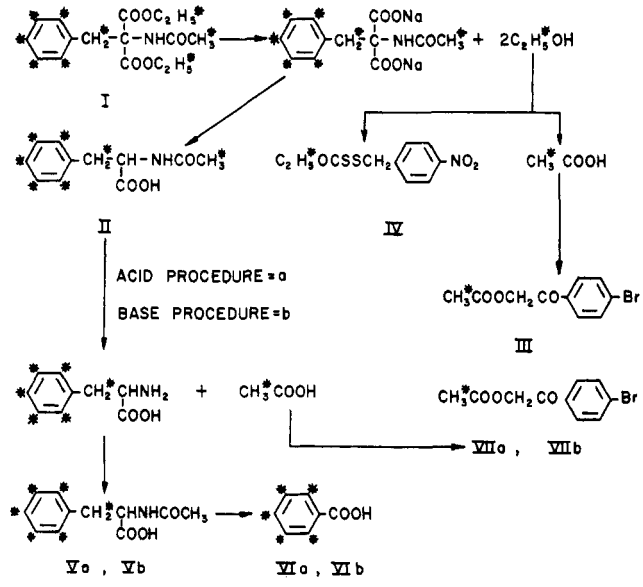
A need for tritiated *dl*-phenylalanine prompted us to apply the tritium gas exposure technique[3] to diethyl benzylacetamidomalonate, from which the desired product is obtainable by suitable degradations. With this more readily purifiable starting material and with a measure of purification inherent in the chemical degradations, it was hoped to ameliorate the difficulties that arise from trace impurities of high specific activity.[3,4] It also provided an opportunity to supplement available information[5−12] on the distribution of tritium among different classes of hydrogen atoms in tritium-irradiated solids.

SCHEME I



Two samples of tritium-irradiated diethyl benzylacetamidomalonate were purified by methods including solution in methanol, treatment with water vapor, and washing with sodium hydroxide to remove labile hydrogen; recrystallization; and repeated chromatography on activated alumina.

Each purified sample (I) was subjected to the degradation and derivatization, outlined in Scheme I, which was designed to determine activity associated with hydrogen at various positions in the molecule. Asterisks denote hydrogen atoms derived from the starting material. The degradation was straightforward, except perhaps for the reacetylation step yielding Va and Vb. This was included because in preliminary experiments with unlabeled phenylalanine, no benzoic acid could be obtained by permanganate oxidation. A good yield was obtained, however, from acetylphenylalanine. The recovery of ethanol from an aqueous solution as ethyl *p*-nitrobenzylxanthate is a little known but highly recommended procedure.

Following conversion of I to II, acidic a, as well as basic b, conditions were used to remove the acetyl group from N-acetylphenylalanine II, sample 2. This modification was incorporated after it was found that there was a loss of activity by exchange in the acetyl group under conditions of strong alkaline hydrolysis. This was not totally unexpected[13,14] and is seen in the lower specific activity of VIIb *vs.* VIIa in Table I. On the other hand, loss by exchange in the aromatic ring was expected[15] during the conditions of strong acid hydrolysis used to remove the acetyl group. Thus benzoic acid VIa showed much lower activity than VIb. Total recovery of activity was therefore calculated by using the values from both routes of degradation.

The best summation of per cent molar specific activity recovered is obtained from IV, Vb, and VIIa. This summation could not be made on sample 1 because a comparable acid hydrolysis was not done. For sam-

(1) To whom inquiries should be addressed; University of Chicago, Department of Medicine, Chicago, Ill.

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TABLE I

ACTIVITY OF FRAGMENTS AND DERIVATIVES OF TRITIUM-IRRADIATED DIETHYL BENZYLACETAMIDOMALONATE

	Activity			
	Sample 1		Sample 2	
Compd.	μc./mmole	%	μc./mmole	%
Diethyl benzylacetamidomalonate (I)	346		28.4	
N-Acetylphenylalanine (II)	311	89.7	26.0	91.7
p-Bromophenacyl acetate (III)	3.06	1.8[a]	0.24	1.7[a]
Ethyl *p*-nitrobenzylxanthate (IV)	10.5	6.1[a]	0.85	6.0[a]
N-Acetylphenylalanine (Va)			18.8	66.2
N-Acetylphenylalanine (Vb)	232	66.9	24.6	86.9
Benzoic acid (VIa)			17.3	60.9
Benzoic acid (VIb)	220	63.6	22.5	79.2
p-Bromophenacyl acetate (VIIa)			0.91	3.2
p-Bromophenacyl acetate (VIIb)	6.6	1.9	0.067	0.24
II + IV		95.8		97.7
IV + Vb + VIIa				96.1

[a] Based on twice the specific activity of this derivative.

TABLE II

TRITIUM DISTRIBUTION IN TRITIUM-IRRADIATED DIETHYL BENZYLACETAMIDOMALONATE

		Activity, μc./mg.-atom of H	
Group	Calculation	Sample 1	Sample 2
Aromatic	Vb/5	44	4.5
CH_3 in ethyl	III/3	1.02	0.08
CH_2 in ethyl	(IV–III)/2	3.72	0.30
CH_3 in acetyl	VIIa/3		0.30
CH_2 in benzyl	(Vb–VIb)/2	6	1.1

ple 2, 96.1% of the molar specific activity of the starting material was accounted for (Table I). As a check on the radiochemical purity of diester I, sample 2 was later diluted 500-fold and chromatographed once again on alumina. Recovery of specific activity of the rechromatographed product was only 92.2% of starting activity; thus some doubt is cast on the distribution figures, and the presence of some radiochemical impurity seems likely.

Distribution per atom of hydrogen is given in Table II. By far the greatest portion of the label was incorporated into the aromatic ring. A nonstatistical distribution of tritium in the *ortho*, *meta*, and *para* positions of the ring might well be expected but no differentiation was made in our study. Tritium in the CH_2 group of the benzyl radical is found by difference and can be calculated in a number of ways, most accurately, in our opinion, from Vb and VIb. The ester and acetyl groups show the lowest tritium content. These differences probably reflect differences in the effective target areas presented by the various groupings, as well as differences in the ability of different types of structures to undergo a high degree of excitation without fragmentation.

The difference in distribution of our two samples may have resulted from traces of impurities of high specific activity. The good recovery of molar specific activity in the fragments of our degraded material is some indication, but not of course proof, of purity of the material degraded. However, apart from the question of purity, it may well be that the distribution reflected the physical condition of the material during irradiation, an effect similar to that found by Simon, who irradiated glucose adsorbed on charcoal.[5] Of our samples, the first had been prepared from aqueous solution; the second had been formed as exceedingly small crystals by rapid cooling of a solution in petroleum ether. In view of the influence of solvent on crystal habit[16,17] it may well be that the two samples differed in the character of the surfaces presented to the tritium gas.

Experimental

Melting points were taken on a calibrated Fisher-Johns hot stage. Activities for the later stages of the purification and for the derivatives were determined by measuring the ionization current[18] of a gas prepared by the zinc fusion technique[19]; an Applied Physics Corp. vibrating reed electrometer and calibrated ion chambers were used. The degradation of sample 1 is described, except where noted. The procedures for sample 2 were essentially similar.

Tritiation and Purification of Diethyl Benzylacetamidomalonate. Sample 1.—A 498-mg. sample of diethyl benzylacetamidomalonate, recrystallized from water, was treated with 6.98 c. of tritium for 9.84 days in the usual manner.[3] The crude product was dissolved twice in succession in 5 ml. of methanol followed each time by removal of the solvent *in vacuo*. After one recrystallization from petroleum ether (b.p. 60–90°) its activity was 260 μc./mg. A portion of the material after dilution 23.6 times with carrier was chromatographed, using chloroform–carbon tetrachloride on activated alumina and discarding the first and last cuts, which had high specific activities. The middle cuts were combined and rechromatographed on alumina. The four center cuts from this column were recrystallized separately from petroleum ether until three of them had approximately the same activity. These were combined, recrystallized, and assayed; they were recrystallized twice more, without change of activity, which remained 1.13 μc./mg. or 346 μc./mmole. This would be equivalent to 26.6 μc./mg. for the undiluted sample.

Sample 2.—Diethyl benzylacetamidomalonate (993 mg.), recrystallized from petroleum ether, was treated with 6.44 c. of tritium for 5.0 days in the usual manner.[3] Before exposure to air, the sample was given three successive treatments with water vapor followed by evacuation. The sample was taken up in toluene, washed with 1 *N* sodium hydroxide, recovered, and recrystallized from petroleum ether and from water containing 5% ethanol to an activity of 13 μc./mg. About 80% of the initial activity was lost in these steps. The sample was then chromatographed using chloroform–carbon tetrachloride on alumina and the middle cuts were collected and recrystallized from petroleum ether to an activity of 9.5 μc./mg. A second chromatography with collection of the middle cuts gave a product with an activity of 9.0 μc./mg. For degradation this product was diluted 97.4-fold to an activity of 0.092 μc./mg., or 28.4 μc./mmole.

Later, upon dilution of a small portion of the undiluted product 500-fold followed by chromatography on alumina, center cuts with activity equivalent to 8.3 μc./mg. were obtained.[20]

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(20) A referee has called our attention to the possibility that this decrease in activity may be the result of exchange with traces of exchangeable hydrogen on the alumina. Our experimental data do not exclude this possibility, because activity recoveries from the column were less than 100%. Exchange presumably did occur during the first chromatography of sample 1, from which an astonishingly small fraction of the starting activity was recovered in the solid; only about 2% additional was removed from the material by 1 *N* sulfuric acid and 1 *N* sodium hydroxide. Alternatively much exchangeable hydrogen was rapidly removed from the unchromatographed material by 1 *N* aqueous sodium hydroxide; hence such a treatment was included for sample 2. In the chromatography in question, exchange on the column was not the sole factor, because first and last cuts had higher specific activity than the center cuts and over-all recovery of activity correlated well with weight recovery.

dl-**N-Acetylphenylalanine (II).**—Following a modification of two procedures[21,22] a distilling flask charged with 382 mg. (1.25 mmoles) of I and 2.0 ml. of 10% sodium hydroxide was stoppered and allowed to stand at room temperature for 5 days, after which time ester hydrolysis was complete. The solvent, containing the labeled ethyl alcohol, was distilled without heating into a cold trap under vacuum. To the residue were added 1.7 ml. of 3 *N* hydrochloric acid and 2.0 ml. of water, and the solution was refluxed for 1.5 hr. The hot, acidic solution was filtered, the flask was washed with 2.0 ml. of water, and the combined solutions were evaporated in a stream of air to about 2 ml. Overnight refrigeration deposited white granular crystals of N-acetylphenylalanine which were recrystallized from water to m.p. 145–146°, in a yield of 229 mg. (86%).

p-**Bromophenacyl Acetate (III).**—To one-half of the aqueous alcohol distilled after hydrolysis of diester I, there were added 267 mg. of potassium permanganate, 141 mg. of magnesium sulfate, and 2 ml. of water. The reaction mixture was allowed to stand at room temperature for 20 hr. Upon addition of 1.5 ml. of 48% hydrobromic acid and sodium bisulfite a clear colorless solution resulted. The solvent, containing the labeled acetic acid, was distilled with gentle heating under vacuum into a cold trap. The distillate was adjusted to pH 5–6 with sodium carbonate, 202 mg. of *p*-bromophenacyl bromide and 4 ml. of ethanol were added, and the solution was refluxed for 1.5 hr. A small amount of water was added and the solution was refrigerated to give crude *p*-bromophenacyl acetate, which was recrystallized from petroleum ether to m.p. 83–84°.

Ethyl *p*-Nitrobenzyl Xanthate (IV).—To the second half of the aqueous alcohol distilled after hydrolysis of I, the following were added with cooling: 1.5 g. of potassium hydroxide, 0.5 ml. of acetone, and 0.5 ml. of carbon disulfide.[23] After shaking for 10 min., the yellow precipitate was extracted into 10 ml. of acetone, and 193 mg. of *p*-nitrobenzyl chloride was added to the extract. The potassium chloride which formed after warming gently for a few minutes was removed by filtration, the solvent was evaporated, and the residue was taken up in 8 ml. of benzene. The extract was washed with 10-ml. portions of 10% sodium hydroxide, the first containing 8 drops of mercaptoacetic acid. After drying over calcium chloride, the benzene was evaporated leaving a residue which was recrystallized from petroleum ether. Long yellow needles of ethyl *p*-nitrobenzylxanthate, m.p. 60–61°, 121 mg., resulted. An unlabeled sample prepared in the same manner was analyzed.

Anal. Calcd. for $C_{10}H_{11}NO_3S_2$: C, 46.68; H, 4.31. Found: C, 46.90; H, 4.33.[24]

Deacetylation of *dl*-N-Acetylphenylalanine (II). Base Procedure b.—A solution of 201 mg. (0.971 moles) of II in 2.0 ml. of 10% sodium hydroxide was refluxed for 5 hr.; after acidification with 1.0 ml. of 48% hydrobromic acid, the solvent containing the labeled acetic acid was distilled into a cold trap under vacuum. The residue was taken up in 6 ml. of water, the solution was filtered, and the filtrate was reduced in volume in an air stream and was brought to pH 5 with ammonia. On refrigeration, *dl*-phenylalanine precipitated and was recrystallized several times from aqueous ethanol containing a few drops of benzene. The final white product weighed 49 mg. (31%) with m.p. 240–245° dec.

p-**Bromophenacyl Acetate (VIIb).**—The distillate from the above experiment was adjusted to pH 5–6 with sodium carbonate, and the *p*-bromophenacyl acetate was prepared as described for III. A sharp separation from contaminating *p*-bromophenacyl bromide was effected by chromatography on 9 g. of activated alumina using 1:1 v./v. benzene–hexane as eluent. Recrystallization from petroleum ether eventually gave 34.7 mg. of pure white needles, m.p. 83–84°.

Deacetylation of *dl*-N-Acetylphenylalanine (II). Acid Procedure a.—This acid hydrolysis was done only on sample 2, from which these data were taken. To 450 mg. of *dl*-N-acetylphenylalanine was added 2.0 ml. of 48% hydrobromic acid, and the solution was refluxed for 2 hr. After cooling, 1 ml. of concentrated ammonium hydroxide was added and the still-acidic solution was submitted to vacuum distillation into a cold trap. The *dl*-phenylalanine was isolated from the residue as described above. From the distillate *p*-bromophenacyl acetate VIIa was prepared and purified as described for VIIb.

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dl-**N-Acetylphenylalanine (Vb).**—*dl*-Phenylalanine (51 mg.) was reacetylated by the method of du Vigneaud,[25] using acetic anhydride in base with cooling. The product was recrystallized several times from water to give 50 mg. (79%) of *dl*-N-acetylphenylalanine, m.p. 149–151°. The sample was quantitatively diluted with carrier.

Benzoic Acid (VIb).—A solution of 381 mg. (1.84 mmoles) of Vb, 2.39 g. of potassium permanganate, 1.0 ml. of 10% sodium hydroxide, and 7.0 ml. of water was refluxed for 2 hr. The reaction mixture was filtered, and the filtrate was acidified and extracted with ether. Evaporation of the ether followed by sublimation of the residue yielded 71 mg. (32%) of fine, long white needles of benzoic acid, m.p. 122°.

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Two Abbreviated Syntheses of Flavones and Flavone Analogs

L. L. WOODS AND JOHN SAPP[1]

Division of Natural and Physical Sciences, Texas Southern University, Houston, Texas 77004

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Two abbreviated methods for the synthesis of flavones and their analogs are described in this report and are elaborations of a procedure developed previously.[2]

The direct condensation of phenols with malonic acid under the influence of trifluoroacetic acid to give the flavones, none of which are naturally occurring,[3] listed in Table I provides an effective one-step method to obtain such substances in good yield. The general course of the reaction may be adequately visualized from the specific equation given for the preparation of compound **1** in Chart I under method A.

Substitution of malononitrile for malonic acid provides an alternate route to the synthesis of flavones but the method cannot, in the strictest sense, be considered to be a one-step procedure as shown in Chart I, method B, for the preparation of compound **5**; however, two of the five compounds listed in Table I are identical with those prepared by the malonic acid method. Compound **6**, previously reported[2] as having a melting point of 145°, does indeed have such a melting point when recrystallized once from ethyl acetate, but two recrystallizations from ethanol give the value recorded in Table I and confirm the results of Wilson and Daniels.[4]

At the end of Table I the names of the several compounds are given, observing the orienting influences which have been fully discussed previously.[2]

The *p*-nitrobenzoates, prepared by the method of Hickenbottom,[5] of the different compounds synthesized

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