Hofmann degradation. A camphoraceous liquid codistilled with the last traces of water and on isolation was found to contain a band at 6.12 μ but not at 2.9–3.0 μ in the infrared. The new product absorbed nearly two molar equivalents of hydrogen over prereduced Adams catalyst to give cycloöctyl methyl ether, as demonstrated by comparison with an authentic sample.

**Cycloöctyl Methyl Ether.**—The sodium salt of cyclooctanol, prepared in anhydrous toluene, was refluxed for 13 hours with excess methyl iodide. Filtration of the sodium iodide, followed by fractional distillation, yielded crude cycloöctyl methyl ether, b.p. 185–191.5°, in 63% yield. An analytical sample showed b.p. 185–189°, $n^{21}D$ 1.4643.

*Anal.* Calcd. for $C_9H_{18}O$: C, 75.99; H, 12.76. Found: C, 76.27; H, 12.52.

**1,2-Epoxycyclohexyl Methyl Ketone.**—Compound XI[20] (12.8 g., 0.10 mole) was allowed to stand at room temperature for 12 hours with 33% hydrogen peroxide (20 ml., 0.20 mole) and 4 *N* sodium hydroxide (22 ml.) in 200 ml. of methanol. Dilution of the reactants with one liter of water, followed by sixfold extraction with ether, drying, and removal of the ether, gave a yellowish liquid. Distillation yielded 6.5 g. (45%) of colorless epoxide, b.p. 84–90° (15 mm.), $n^{22}D$ 1.4633.

*Anal.* Calcd. for $C_8H_{12}O_2$: C, 68.64; H, 8.63. Found: C, 69.08; H, 8.68.

An impure, red 2,4-dinitrophenylhydrazone, $\lambda_{max}^{CHCl_3}$ 367 mμ, formed but could not be successfully recrystallized. Attempts to prepare a semicarbazone met with failure.

**1,2-Dihydroxycyclohexyl Methyl Ketone (XII).**—A suspension of the epoxide (7.0 g.) in 55 ml. of water containing 15 drops of concentrated sulfuric acid was held at steam-bath temperatures for two hours. The mixture was then neutralized with sodium carbonate and extracted with 400 ml. of ether in six portions. Isolation of the product by the usual procedure, followed by distillation, gave 5.3 g. (68%) of colorless XII, b.p. 103.5–105.0° (2.0 mm.), m.p. 53° after recrystallization from ether–pentane.

*Anal.* Calcd. for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.85; H, 8.80.

The glycol was partly resinified on exposure to sirupy phosphoric acid for one hour at 100°, and completely resinified on similar treatment at 130°. In the former case, some starting material was recovered.

**1,2-Dibromocyclohexyl Methyl Ketone (XIII).**—Following a literature procedure, a chilled solution of XI in chloroform reacted with the required amount of bromine, but evaporation of the solvent at low temperatures[24] gave rise to a dark, viscous solution from which no appreciable amount of the desired dibromide could be isolated.

A somewhat more satisfactory procedure involved bromination in acetic acid solution, neutralization of the reaction mixture with aqueous alkali, extraction of the dibromide with ether, decolorizing the ether solution with charcoal, and finally evaporation to dryness. In this way 4.0 g. of XI gave 2.1 g. (23%) of white dibromide, m.p. 43.0–43.5° (lit. value 48°).[24] The dibromide darkened fairly rapidly, even in the absence of light.

**Pyridine Treatment of Dibromide XIII.**—A sample of XIII (0.30 g.) was refluxed in pyridine (3 ml.) under nitrogen for one hour. Acidification and steam distillation yielded, after the usual work up, 30 mg. (23%) of a liquid very nearly identical in infrared and ultraviolet spectra to an equimolar mixture of acetophenone and 1-cyclohexenyl methyl ketone. Attempts to isolate a diene intermediate were unrewarding.

(24) R. B. Wagner, THIS JOURNAL, **71**, 3214 (1949).

ITHACA, N. Y.

---

[CONTRIBUTION OF THE CHEMICAL LABORATORIES OF BRANDEIS UNIVERSITY AND OF SARAH LAWRENCE COLLEGE]

# Asymmetry in the Reaction of *l*-Menthol with β-Phenylglutaric Anhydride, an Analog of Citric Acid.[1] Preparation of Optically Active Pyrrolidides of β-Phenylglutaric Acid

BY ROLF ALTSCHUL, PHILIP BERNSTEIN AND SAUL G. COHEN[2]

RECEIVED DECEMBER 29, 1955

β-Phenylglutaric anhydride, taken as a model for citric acid and for other molecules of type Ca, b, d, d shows asymmetry in its reaction with *l*-menthol at 155°, leading to the diastereomeric mono-*l*-menthyl esters in unequal (54:46%) yields. Thus the enzymatic reactions of substrates of type Ca, b, d, d may take place at the two sites d at unequal rates not necessarily because of three point contact between the compound and the optically active enzyme, but possibly because of reaction *via* diastereomeric transition states. Separation of the diastereomeric mono-*l*-menthyl-β-phenylglutarates is a process similar to resolution, and reaction of the individual esters with lithium pyrrolidide leads to the optically active enantiomorphic mono-pyrrolidides of β-phenylglutaric acid.

Experiments with $C^{14}$-labeled citric acid prepared both biochemically[3] and from (−)γ-chloro-β-carboxy-β-hydroxybutyric acid,[4] $(-)ClCH_2C(OH)(COOH)CH_2COOH$, have confirmed that the enzymatic degradation of citric acid, $HO_2CCH_2C(OH)(CO_2H)CH_2CO_2H$, to α-ketoglutaric acid, $HO_2C\text{-}CO\text{-}CH_2CH_2CO_2H$, *via* aconitic and isocitric acids proceeds asymmetrically, the enzyme distinguishing between the two carboxymethyl groups. Similar specificity may be observed in other enzymatic processes—the conversion of serine, $CH_2(OH)CH(NH_2)CO_2H$ to glycine[5] possibly *via* the symmetric aminomalonic acid, and the stereospecific reduction[6] of the symmetric deuteroacetaldehyde, $CH_3CD{=}O$ by reduced diphosphopyridine nucleotide. This kind of stereochemical specificity is commonly attributed to a three point contact between substrate and enzyme.[7] These reactions, however, may be interpreted in terms of different rates of formation of diastereomeric transition states, although such analysis cannot disprove the three point contact mechanism.

Formation of diastereomers is very common and, requiring the formation of at least two centers of asymmetry in a transition state or product, may occur under conditions which include the following: (1) two reactants, each containing a center of asymmetry, combine, as in the common method of resolution of enantiomorphs *via* diastereomers.

(1) While this work was in progress, the related study of the reaction of β-phenylglutaric anhydride with *l*-α-phenylethylamine was reported, P. Schwartz and H. E. Carter, *Proc. Nat. Acad. Sci.*, **40**, 499 (1954).

(2) Brandeis University, Waltham, Massachusetts.

(3) V. R. Potter and C. Heidelberger, *Nature*, **164**, 180 (1949).

(4) P. E. Wilcox, C. Heidelberger and V. R. Potter, THIS JOURNAL, **72**, 5019 (1950).

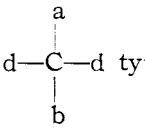
(5) D. Shemin, *J. Biol. Chem.*, **162**, 297 (1946).

(6) F. A. Loewus, F. H. Westheimer and B. Vennesland, THIS JOURNAL, **75**, 5018 (1953).

(7) A. G. Ogston, *Nature*, **162**, 963 (1948).

(2) Two reactants, neither containing centers of asymmetry, form a product containing two such centers, as in the stereospecific addition of halogen to 1,2-disubstituted olefins.[8] (3) Two reactants, one of which contains a center of asymmetry lead to products or transition states containing an additional center of asymmetry. These diastereomers may be formed at unequal rates and in unequal quantities and may lead to partial asymmetric synthesis and optical activity. Examples include (a) a variety of additions to asymmetric carbonyl compounds[9-11]; (b) partial asymmetric reduction of carbonyl compounds by an optically active Grignard reagent[12] or by optically active aluminum alkoxide[13]; (c) the classical partial asymmetric decarboxylation of the brucine salt of methylethylmalonic acid.[14a,b]

The reactions of the compounds citric acid, amino malonic acid and 1-deutero-acetaldehyde with enzymes or simple optically active reagents fall within the third group and are related to reactions 3,b and c. These materials may be represented by a formula of the



type in which the two similar groups d are carboxymethyl in citric acid, carboxyl in aminomalonic acid and the doubly bonded oxygen in acetaldehyde. In each case the two similar groups will have equal chemical reactivity toward non-asymmetric reagents. However, the two groups d are not equivalent stereochemically since conversion of one group *d* to *e* either by an intramolecular reaction or by reaction with an external reagent, leads to one (+) enantiomorph of C,a,b,d,e while conversion of the other group *d* leads to the second (−) enantiomorph. If the reaction at a site d, in which d is being converted to e, is with an optically active reagent (−), then the transition state at the one site will have the properties of a (+,−) diastereomer and the transition state at the other site will have the properties of a (−,−) diastereomer, and the diastereomeric transition states and the resulting products may well be formed at different rates.

**Reaction of β-Phenylglutaric Anhydride with *l*-Menthol.**—It seemed desirable to us to examine reactions of reasonably well-understood mechanism of a molecule C,a,b,d,d with an optically active reagent. β-Phenylglutaric anhydride (I) was chosen for its two potential carboxymethyl groups, in analogy with citric acid. Its reactions with *l*-menthol (II) at the two carbonyl groups of the anhydride linkage would produce diastereomeric monomenthyl esters, probably at unequal rates. Subsequent treatment of the individual esters with an inactive amine would lead to optically active enantiomorphic mono-amides (Chart I).

Schwartz and Carter,[1] also interpreting the citric acid problem in terms of the C,a,b,d,d model, studied the reaction of β-phenylglutaric anhydride with *l*-α-phenylethylamine and concluded that the diastereomeric monoamides were formed in 60:40 ratio. Their introduction of the term "*meso* carbon atom" was subsequently criticized.[15]

In one experiment (run 3), the product of reaction of β-phenylglutaric anhydride (I) with slightly less than one mole of *l*-menthol (II) at 155° was crystallized from benzene into four fractions (96% recovery) and each was examined for weight, melting point, specific rotation, neutralization equivalent and content of alkali insoluble di-*l*-menthyl-β-phenylglutarate (III). From the neutralization equivalent and the content of III, the yields of mono-menthyl-β-phenylglutarate (IV) and of I were calculated. The contents of the three components accounted for 95.5% of the β-phenylglutaric anhydride. Pure samples of the diastereomeric monomenthyl esters were obtained: (A) m.p. 173–174°, $[\alpha]^{25}D$ − 59.0°, from fraction 1; and (B) m.p. 112–113°, $[\alpha]^{25}D$ − 36.9°, from fractions 3 and 4; from their specific rotations and that of the dimenthyl ester, m.p. 105–106°, $[\alpha]D^{25}$ − 67.5°, and from the content of I, the yields of diastereomers A and B were calculated. The yields of these diastereomeric esters formed at the two carbonyl sites in I were in the ratio 54:46; 4.5% of the starting material was lost in manipulation, and if all of it were diastereomer IVB, which is unlikely, the yield of IVA would still exceed that of IVB. This confirms the results of Schwartz and Carter; a model compound of type Ca,b,d,d may react with a simple asymmetric reagent at sites d to form the diastereomeric products in unequal yield. The data are summarized in Table I.

TABLE I

REACTION OF β-PHENYLGLUTARIC ANHYDRIDE WITH *l*-MENTHOL, 155°, 9.5 HOURS

| Compound[a] | | G. | Mmole | % |
|---|---|---|---|---|
| I | Initial | 8.84 | 46.5 | .. |
| I | Final | 0.58 | 3.04 | 6.5 |
| III | Product | 0.87 | 1.78 | 3.8 |
| IVA | Product | 7.41 | 21.40 | 46.0 |
| IVB | Product | 6.30 | 18.20 | 39.2 |

[a] I, β-phenylglutaric anhydride; III, di-*l*-menthyl-β-phenylglutarate; IV, mono-*l*-menthyl-β-phenylglutarate.

Whether unequal yields in this case are due to unequal rates of formation, or to equilibration of the two products to form a higher yield of the more stable product, or to unequal rates of conversion of the mono-esters to the di-ester III, is not clear. When the reaction of β-phenylglutaric anhydride and menthol was repeated at 155°, but run for 24 hours instead of 9.5 hours, the yield of the dimenthyl ester III rose from 3.8 to 16.2%. Since titrations during the course of the first run indicated that the menthol was at least 95% consumed during the first three hours, both menthyl groups which appear in dimenthyl ester III on prolonged

(8) G. W. Wheland, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y. 2nd Edition, 1949, pp. 290–298.

(9) E. Fischer, *Ber.*, **27**, 3231 (1894); **22**, 370 (1889).

(10a) A. McKenzie, *J. Chem. Soc.*, **85**, 1249 (1904).

(10b) V. Prelog, A. Furlenmeier, D. F. Dickel and W. Keller, *Helv. Chem. Acta*, **36**, 308 (1953).

(11) D. J. Cram and F. A. A. Elhafez, THIS JOURNAL, **74**, 5828 (1952).

(12) H. S. Mosher and E. LaCombe, *ibid.*, **72**, 3994 (1950).

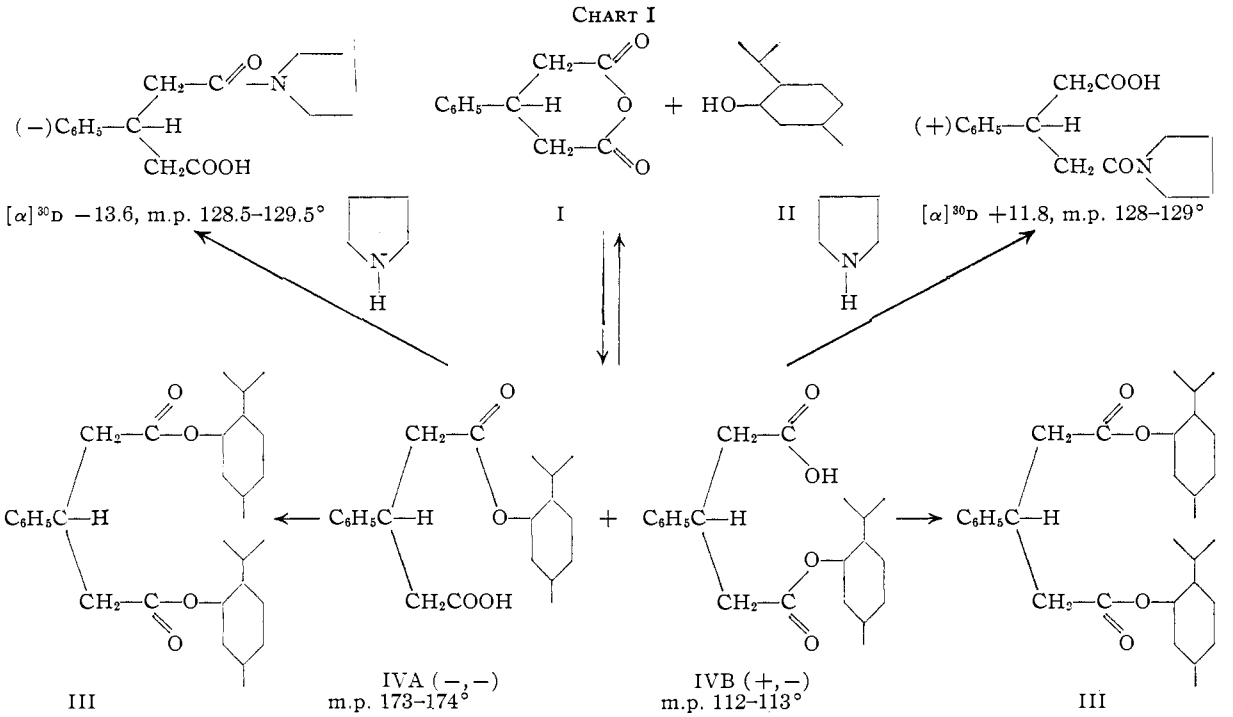
(13) W. E. Doering and R. W. Young, *ibid.*, **72**, 631 (1950).

(14a) W. Marckwald, *Ber.*, **37**, 349 (1904).

(14b) J. Kenyon and W. A. Ross, *J. Chem. Soc.*, 3407 (1951); 2307 (1952).

(15) M. L. Wolfrom, *Proc. Nat. Acad. Sci.*, **40**, 794 (1954).

CHART I




heating had been incorporated previously in monomenthyl ester IV. This may indicate that the reaction of I and II to form IVA and IVB is reversible, providing opportunity both for equilibration of IVA with IVB and for their conversion to dimenthyl ester III by slow reaction with the small equilibrium concentration of menthol (see Chart I).

In accord with this interpretation, and since the formation of dimenthyl ester III causes an undesired complication in the polarimetric analysis, a run was made in which two moles of anhydride I was treated with one mole of menthol at 155° for six hours, samples being titrated and measured polarimetrically as a function of time. The menthol had reacted essentially completely when the first sample was taken at 98 minutes, and we were unable to isolate any dimenthyl ester III. The large excess of anhydride minimized the formation of dimenthyl ester. The optical rotations again indicated that diastereomer IV A was formed in 54% yield, IV B in 46% yield (run 5, Experimental Part).

**Optically Active Pyrrolidides of β-Phenylglutaric Acid.**—Monocyclohexyl-β-phenylglutarate was prepared from the anhydride and cyclohexanol and its reaction with pyrrolidine was examined under varied conditions. The monopyrrolidide was obtained from the ester by treatment with a solution of lithium in pyrrolidine, but the yield was low. This meant that it would not be feasible to demonstrate asymmetry in the reaction of *l*-menthol with β-phenylglutaric anhydride by treatment of the product—the mixture of diastereomeric monomenthyl esters—with pyrrolidine, followed by search for optical activity in the pyrrolidide. Unequal yields of the enantiomorphic pyrrolidides from the two diastereomeric esters would be possible and likely and lead to activity in the product.

The formation of the diastereomeric monomenthyl esters of β-phenylglutaric acid and their separation by fractional crystallization corresponds quite closely to resolution of a *d,l* material *via* diastereomers, except that removal of the optically active reagent, *l*-menthol, by hydrolysis, would lead to an inactive material, the two carboxymethyl groups being identical. Perhaps this procedure might be called a "pseudo resolution." However, replacement of the *l*-menthyl group by amino instead of hydroxyl restores the analogy to resolution. Optically active, enantiomorphic monopyrrolidides, m.p. 128.5–129.5°, were formed in this way, by isolation of the individual mono-*l*-menthyl diastereomers, followed by treatment of each with lithium pyrrolidide, rather than by formation of the *racemic*-pyrrolidide and resolution of it. The racemic pyrrolidide was prepared by reaction of β-phenylglutaric anhydride with pyrrolidine and by treatment of monocyclohexyl β-phenylglutarate with lithium pyrrolidide, m.p. 108–109°. The melting point of a mixture of the enantiomorphs was identical with that of the *d,l*-material. The infrared spectrum of the *racemic*-pyrrolidide was identical with those of the optically active enantiomorphs, with absorption bands at 1710 cm.$^{-1}$, a shoulder at 1615 cm.$^{-1}$ on a strong band at 1575 cm.$^{-1}$ and a broad band at 1410–1450 cm.$^{-1}$.

In the course of this work the *d,l*-monopiperidide was prepared from β-phenylglutaric anhydride and piperidine and the *d,l*-monomethyl ester was prepared from the anhydride and methanol.

## Experimental

Melting points are uncorrected. Elementary microanalyses were performed by Dr. S. M. Nagy, Massachusetts Institute of Technology.

Optical rotations were measured on a Zeiss Jena Polarimeter graduated to 0.05°. Unless otherwise indicated rotations were measured in dioxane at about 5% concentration in a two-decimeter tube. Observed rotations, α, were in the range of 3 to 5°; at least six readings were made

of each rotation and the average error was about ±0.012°. This leads to an error of not more than 1% in the calculated yields of the diastereomers, run 5. The specific rotations were calculated: $[\alpha]^{T}D\ 100\alpha/l.c.$

Infrared spectra of the monopyrrolidides were determined in chloroform solution on a Model 21 Perkin–Elmer double beam spectrophotometer.

**β-Phenylglutaric anhydride**[16a] was prepared by addition of diethylmalonate, 128 g. (0.8 mole), to ethyl cinnamate, 144 g. (0.82 mole), in the presence of 2 g. of sodium dissolved in 50 ml. of absolute ethanol, followed by saponification and decarboxylation. The free acid was not isolated but the product resulting from decarboxylation was boiled for one hour with 450 cc. of acetic anhydride, concentrated, crystallized from benzene, and dried *in vacuo* at 55°, 55 g. (33% yield). The melting point was low, 96–97°, reported 105°; equivalent weight 104.5, calculated 95.1. A sample, 0.524 g., was dried *in vacuo* at 105° for 2.5 hours, losing 0.0507 g. of solvent, the remainder melting at 105–106°, neutralization equivalent, 95.4. The total product was treated similarly and brought to the correct melting point and neutralization equivalent. In subsequent work it was found that crystallization from ethyl acetate led directly to a product with the correct properties.

**Run 3.**—β-Phenylglutaric anhydride, 10.31 g. (0.0542 mole) and *l*-menthol (Merck, m.p. 40–43°, $[\alpha]^{25}$ −47.4°) 8.24 g. (0.0527 mole), were melted in a stoppered test-tube, stirred to homogeneity and heated at 154 ± 2° for 9.5 hours. Samples were removed at intervals, *ca.* 0.3 g., dissolved in 10 cc. of C.P. acetone, treated with a substantial portion of the theoretical quantity of standard alkali (0.1012 *N*) and 30 cc. of water, warmed to assure complete hydrolysis of the anhydride, and titrated to phenolphthalein end-point. The progress of the esterification was as follows, the first number in each pair indicating the time of reaction in minutes, the second, the per cent. consumption of *l*-menthol: 120, 88.3; 200, 95.0; 300, 96.9; 490, 95.5; 570, 98.2. The remainder of the product, 15.88 g., 85.7% of the original, was crystallized from benzene leading to 95.9% recovery in four fractions: Fr. 1, 7.00 g., m.p. 168–174°, $[\alpha]^{25}D$ −57.6° (*c* 5.47); Fr. 2, 2.45 g., m.p. 118–140°, $[\alpha]^{25}D$ −42.5°, (*c* 5.56); Fr. 3, 2.86 g., m.p. 96–106°, $[\alpha]^{25}D$ −34.8° (*c* 5.80); Fr. 4, 2.90-g., m.p. 86–100°. No odor of menthol was detected in any fraction. A sample of each fraction was titrated against standard alkali as described above. The sample of Fr. 1 was very slightly cloudy at the end-point while Frs. 2, 3 and 4 each showed strong turbidity which was converted to a white flaky precipitate by gentle heating. The precipitates were collected quantitatively, and dried *in vacuo*, di-*l*-menthyl-β-phenylglutarate (III). The results of the analysis of the four fractions are as follows, the first figure in each pair being the neutralization equivalent, the second the per cent. content of the dimethyl ester; the neutralization equivalent of the monomenthyl ester is 346.5: Fr. 1, 348, 0.0%; Fr. 2, 337, 5.0%; Fr. 3, 267, 4.8%; Fr. 4, 387, 20.6%.

The remainder of fraction 1 was crystallized three times from benzene leading to mono-*l*-menthyl-β-phenylglutarate, diastereomer, A, m.p. 173–174°, $[\alpha]^{25}D$ −59.0° (*c* 5.70). An additional crystallization from acetic acid did not affect these data. *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.79; H, 8.73; neut. equiv., 346.5. Found: C, 72.77; H, 8.67; neut. equiv., 347.0.

The remainder of fractions 3 and 4 were combined, dissolved in acetone, neutralized with 2 *N* sodium hydroxide and warmed on the water-bath to evaporate much of the acetone and cause the di-*l*-menthyl-β-phenylglutarate to crystallize. This product was collected, dried *in vacuo* and crystallized from acetic acid; m.p. 105–106°, $[\alpha]^{25}D$ −67.5° (*c* 5.57). *Anal.* Calcd. for $C_{31}H_{49}O_4$: C, 76.81; H, 9.98. Found: C, 76.56, 76.82; H, 10.06, 10.07.

The aqueous filtrate was acidified strongly with concentrated hydrochloric acid and extracted with three portions of ether. The extracts were dried and evaporated leading to a mixture of mono-*l*-menthyl-β-phenylglutarate, largely diastereomer B, and β-phenylglutaric acid. Diastereomer B was isolated by fractional crystallization from benzene–petroleum ether and glacial acetic acid–water; m.p. 112–113°, $[\alpha]^{25}D$ −36.9 (*c* 5.45). An additional crystallization from acetone–water did not change these values. *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.79; H, 8.73; neut. equiv., 346.5. Found: C, 72.80; H, 8.63; neut. equiv., 347.1.

(16) (a) D. Vorlander, *Ann.*, **320**, 85 (1902); (b) **320**, 93 (1902).

Diastereomer B is readily soluble in cold benzene, ethyl alcohol, acetic acid and dioxane, and slightly soluble in petroleum ether. Diastereomer A is insoluble in all these solvents at room temperature, except dioxane, in which its solubility is about 7 g./100 cc. The dimenthyl ester is soluble in all these solvents except acetic acid from which it may be crystallized.

Specific rotation of fractions 1–4, corrected for content of β-phenylglutaric anhydride are: Fr. 1, $[\alpha]^{25}D$ −57.6° (no correction); Fr. 2, $[\alpha]^{25}D$ −44.1° (*c* 5.36); Fr. 3, $[\alpha]^{25}D$ −41.0° (*c* 4.92).

The contents of β-phenylglutaric anhydride (I), dimenthyl β-phenylglutarate (III), and monomenthyl β-phenylglutarate (IVA) and (IVB) in the four fractions are: fraction 1, 6.56 g. IVA, 0.44 g. IVB; fraction 2, 0.08 g. I, 0.12 g. III, 0.59 g. IVA, 1.65 g. IVB; fraction 3, 0.39 g. I, 0.14 g. III, 0.26 g. IVA, 2.03 g. IVB; fraction 4, 0.11 g. I, 0.61 g. III, 2.18 g. IVB.

**Run 7.**—β-Phenylglutaric anhydride, 3.799 g. (0.0200 mole) and *l*-menthol, 3.015 g. (0.0193 mole), were heated in an evacuated sealed tube at 155 ± 3° for 24.3 hours. The product was dissolved in 30 ml. of acetone; 3.5 ml. of 6 *N* sodium hydroxide was added, followed by 60 ml. of water and an additional 0.3 ml. of the alkali to turn phenolphthalein. The suspension was digested on the water-bath for 30 minutes, and the solid was collected and dried *in vacuo*, di-*l*-menthyl-β-phenylglutarate, m.p. 105–107°, 1.55 g. (16.2% yield).

**Run 5.**—β-Phenylglutaric anhydride, 6.86 g. (0.0361 mole) and l-menthol, 2.82 g. (0.0180 mole) were heated at 155 ± 2°. Samples were withdrawn at intervals for titration and determination of optical rotation. The titrated solutions were free of solid indicating absence of di-*l*-menthyl-β-phenylglutarate. The titrations indicated slightly more than 100% consumption of acid based on menthol because a small amount of anhydride sublimed to the top of the reaction flask and was not taken in the aliquots. The specific rotations were corrected for excess optically inactive β-phenylglutaric anhydride, this in turn being corrected for sublimed anhydride as indicated by the titrations. The values were constant at 120, 155 and 362 minutes, $[\alpha]^{25}D$ −31.6° ± 0.1 (observed), $[\alpha]^{25}D$ −48.8° ± 0.1 (corrected), 53.7 ± 0.4% diastereomer IVA, 46.3 ± 0.4% diastereomer IVB.

**Monocyclohexyl β-Phenylglutarate.**—β-Phenylglutaric anhydride, 4.0 g. (0.021 mole) and cyclohexanol, 2.0 g. (0.020 mole), were heated for 9 hours in an oil-bath at 155°. The product was crystallized from methanol–water and from hexane, 4.6 g. (76% yield), m.p. 88–89°. *Anal.* Calcd. for $C_{17}H_{22}O_4$: C, 70.31; H, 7.64; neut. equiv., 290. Found: C, 70.69; H, 7.56; neut. equiv., 286, 287.

**β-Phenylglutar-monopyrrolidide.**—Pyrrolidine, 1.5 g., (0.021 mole) was added dropwise to a solution of 4 g. (0.021 mole) of β-phenylglutaric anhydride in 25 ml. of benzene at 50°. After 30 minutes a drop of solution was diluted with ethyl acetate, crystals were obtained and were added to the reaction solution which was refrigerated. The product was filtered, the filtrate was concentrated and a second fraction was crystallized from ethyl acetate, total, 2.55 g. (45% yield), m.p. 108–109°. *Anal.* Calcd. for $C_{15}H_{19}O_3N$: N, 5.36; neut. equiv., 261. Found: N, 5.56; neut. equiv., 258, 259.

**β-Phenylglutar-monopiperidide.**—Piperidine, 1.78 g. (0.021 mole) was added dropwise to a solution of 4 g. (0.021 mole) of β-phenylglutaric anhydride in benzene at room temperature, an exothermic reaction occurring. After one hour, the solution was concentrated *in vacuo* at 70°, the product was collected and crystallized from ethyl acetate, 3.3 g. (57% yield), m.p. 138–139°, reported[16b] 120° from ethanol–water. *Anal.* Calcd. for $C_{16}H_{21}O_3N$: N, 5.09; neut. equiv., 275. Found: N, 5.05; neut. equiv., 274.

**Monopyrrolidide from Monocyclohexyl-β-phenylglutarate.**—Treatment of the ester with an equal weight (4 moles) of pyrrolidine (a) in dimethylformamide at 90° for 2 hours, (b) without solvent at 70° for 17 hours and (c) in an equal weight of water at 100° for 14 hours, led in each case to recovery of the ester in 85–95% yield.

Lithium, 0.1 g. (0.03 mole), was heated in 15 g. (0.21 mole) of pyrrolidine under reflux for 24 hours, nearly all of the lithium dissolving. Monocyclohexyl-β-phenylglutarate, 1 g. (0.0035 mole) was added and the solution was allowed to stand at room temperature for 23 hours. It was acidified

with cold 6 *N* hydrochloric acid, extracted with chloroform, dried and concentrated, leading to the monopyrrolidide, 0.155 g. (17% yield), m.p. 107–108° (from ethyl acetate), mixed m.p. with an authentic sample, 107–108°.

**Optically Active β-Phenylglutar-monopyrrolidide.** (a).—Pyrrolidine was distilled from sodium. Lithium, 0.1 g. (0.03 mole), was boiled in 15 g. of this pyrrolidine under reflux with exclusion of moisture for 40 hours. *l*-Menthyl-β-phenylglutarate (IVA), 1.5 g. (0.0043 mole), was added and the solution was allowed to stand at room temperature for 6 hours. The solution was cooled, acidified to *p*H 1 with 6 *N* hydrochloric acid and filtered; menthol was collected and purified by sublimation, 0.36 g. (54% yield), m.p. 35–36°. The filtrate was extracted with eight 15-ml. portions of chloroform, which were dried and concentrated. The residue was crystallized from ethyl acetate, 0.47 g. (41% yield), m.p. 128.5–129.5°, $[\alpha]^{30}D$ −13.6° (*c* 2.18, absolute ethanol). *Anal.* Calcd. for $C_{15}H_{19}O_3N$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.62; H, 7.23; N, 5.37. In two other experiments run under similar conditions, the yields were lower, 16 and 19%.

(b).—*l*-Menthyl-β-phenylglutarate, diastereomer IVB was treated with lithium pyrrolidide in essentially the same way, leading to menthol and to the enantiomorphic pyrrolidide, 0.175 g. (17% yield), m.p. 128–129°, $[\alpha]^{30}D$ +11.8 (*c* 2.34, absolute ethanol). The compound was crystallized a second time from ethyl acetate and analyzed. Found: C, 68.54; H, 7.24; N, 5.57; a mixed m.p. with an approximately equal amount of the first enantiomorph was 108–110°; the racemic material synthesized from the anhydride melted at 108–109°.

**Monomethyl-β-phenylglutarate.**—β-Phenylglutaric anhydride, 2 g., (0.01 mole), was boiled under reflux for 6 hours in 25 ml. of dried methanol. The methanol was removed *in vacuo*, the residue was crystallized from cyclohexane, 2.19 g. (94% yield), m.p. 96–97°. *Anal.* Calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35; neut. equiv., 222. Found: C, 65.09; H, 6.21; neut. equiv., 220, 220.

**Acknowledgment.**—We are pleased to acknowledge generous support of this work by grants from the American Academy of Arts and Science and from the Committee on Research Grants of Brandeis University.

WALTHAM 54, MASS.

---

[CONTRIBUTION FROM THE WESTERN UTILIZATION RESEARCH BRANCH][1]

# Effect of Ascorbic Acid on Polyphenol Oxidase

BY LLOYD L. INGRAHAM

RECEIVED OCTOBER 10, 1955

A study is reported of the effect of ascorbic acid concentration on polyphenol oxidase. This study involves measurement of initial rates of oxidation, amount of ascorbic acid oxidized in 5 minutes, and limiting amount of ascorbic acid oxidized. The conclusion is drawn that ascorbic acid does not activate or inhibit either the rate of oxidation of catechol or the reaction–inactivation of the enzyme.

In many researches on polyphenol oxidase[2–4] ascorbic acid has been assumed to serve only to keep the substrate reduced and not to affect the enzyme directly. However, other papers have reported that ascorbic acid activates[5] polyphenol oxidase (from mushrooms) and also that it inhibits[6] polyphenol oxidase (from potatoes).

Baruah and Swain[6] have carefully studied the effect of copper on the reaction and have come to the conclusion that the apparent activation by ascorbic acid is the result of traces of copper present in solution which catalyzes the aerobic oxidation of ascorbic acid.[7,8]

Baruah and Swain[6] have reported that polyphenol oxidase is inhibited by reaction for two hr. with ascorbic acid in the absence of substrate under nitrogen. However, the experiments described in this paper show that the polyphenol oxidase catalyzed aerobic oxidation of catechol is not inhibited by ascorbic acid. It is possible that there is a slow reaction between free polyphenol oxidase and ascorbic acid which does not occur when the enzyme is protected with oxygen or catechol.

**Test of Inhibition by Ascorbic Acid.**—The report[6] that ascorbic acid is an inhibitor for polyphenol oxidase described an experiment that used Sreerangachar's method[9] (the amount of ascorbic acid oxidized in 5 min.) and potato as a source for the enzyme. This experiment was checked with an identical method on 3 enzyme preparations (A, B and C) prepared from potatoes by the procedure described by Baruah and Swain.[6] With only one of these preparations (A) was it possible to find any inhibition by ascorbic acid. Furthermore, the inhibition of the preparation (A) could not be repeated quantitatively and it foamed badly during the activity determination. It is quite possible that the ascorbic acid in some way affects surface denaturation of the enzyme. When the consumption of oxygen in solution was measured by the rotating platinum electrode,[10] which involves no bubbling, the activity was found to be independent of the ascorbic acid concentration. The results for this enzyme preparation (A) are summarized in Table I. Sreerangachar's method was also used to test the effect of ascorbic acid on polyphenol oxidase from mushrooms. These results, summarized in Table II together with results from the other two potato enzyme preparations (B) and (C), show no effect of ascorbic acid on activity. The inhibition found with preparation A could not be duplicated by adding enough egg albumin to preparations B and C to make them foam.

(1) Agricultural Research Service, U. S. Department of Agriculture, Albany 10, California.

(2) W. H. Miller, M. F. Mallette, L. J. Roth and C. R. Dawson, THIS JOURNAL, **66**, 514 (1944).

(3) I. Asimov and C. R. Dawson, *ibid.*, **72**, 820 (1950).

(4) L. L. Ingraham, J. Corse and B. Makower, *ibid.*, **74**, 2623 (1952).

(5) R. C. Krueger, *ibid.*, **72**, 5582 (1950).

(6) P. Baruah and T. Swain, *Biochem. J.*, **55**, 392 (1953).

(7) A. Weissberger, J. E. Lu Valle and D. S. Thomas, Jr., THIS JOURNAL, **65**, 1934 (1943).

(8) A. Weissberger and J. E. Lu Valle, *ibid.*, **66**, 700 (1944).

(9) H. B. Sreerangachar, *Biochem. J.*, **37**, 653 (1943).

(10) L. L. Ingraham, *Anal. Chem.*, **28**, 1177 (1956).