**α-Chloroacid Chlorides of Substituted Benzilic Acids.**—3-Methylphenyl phenyl-, 3,4-dimethylphenyl phenyl- and 3,4-methylenedioxyphenyl phenylchloroacetyl chlorides[3]: In these cases, distillation under reduced pressure led to decomposition of the final products. They were therefore freed from ether, subjected to high vacuum for 15 min., and employed without further purification.

**Phenylcyclopentylchloroacetyl Chloride.**[4]—Phenylcyclopentylacetic acid (10 g.) gave 9.8 g. (78%) of chloroacid chloride, b.p. 124° (0.5 mm.)

*Anal.* Calcd. for $C_{13}H_{14}Cl_2O$: Cl, 27.58. Found: Cl, 27.80.

**Ester Hydrochlorides of Substituted Hydroxyacids in Table II.** 3-N,N-Dimethylaminopropylisothiouronium chloride hydrochloride was obtained in 83% yield from 3-chloro-N,N-dimethylpropylamine hydrochloride by the method of Albertson and Clinton,[21] m.p. 159–161°.

*Anal.* Calcd. for $C_6H_{17}Cl_2N_3S$: C, 30.77; H, 7.32. Found: C, 30.94, H, 7.16.

**2-N,N-Dimethylaminopropanethiol**[21] was used in the ethereal solution obtained on extraction.

Compounds 110 through 118 in Table II were prepared by identical procedures which may be described by the synthesis of **3-N,N-dimethylaminopropylthiolbenzilate hydrochloride** according to the method of Kolloff, *et al.*[6] To a solution of 8 g. of diphenylchloroacetyl chloride[3] in 50 ml. of anhydrous ether, was added the ethereal extract from the alkaline hydrolysis of 7 g. of 3-N,N-dimethylaminopropyl isothiouronium chloride hydrochloride; an oil precipitated immediately. After refluxing for 1 hr., the reaction mixture was cooled and the ether was decanted. The residual oil was heated on the steam bath for 15 min. with 100 ml. of water and one drop of concd. hydrochloric acid. Upon being cooled and made basic with sodium carbonate, a white solid separated. Several crystallizations from ethanol led to m.p. 85–87°. When hydrogen chloride was passed into a solution of the solid in anhydrous ether, 1.8 g. (19%) of a white solid formed, which melted at 180–182° after several crystallization from ethanol–ether.

Compounds 119, 120 and 121 were prepared from the corresponding α-chloro derivatives by a procedure which may be illustrated for **2-N,N-diethylaminoethyl phenylcyclohexylthiolglycolate hydrochloride**. The α-chlorester hydrochloride (2.5 g.), dissolved in a minimum amount of water, was refluxed for 2 hr., made distinctly alkaline with sodium carbonate solution, and extracted with ether. The dried ethereal solution was acidified with ethereal hydrogen chloride to give a solid which, when crystallized from a mixture of acetone, methanol, and ether, weighed 2.05 g. (86%) m.p. 174–175.5°.

(21) N. F. Albertson and R. O. Clinton, *J. Am. Chem. Soc.*, **67**, 1222 (1945).

# Derivatives of (−)-*trans*-2,3-Epoxysuccinic Acid and Some of their Biological Effects

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Symmetrical esters and amides as well as the nitrile have been prepared from the mold metabolite, (−)-*trans*-2,3-epoxysuccinic acid. Opening of the oxirane ring in epoxysuccinic acid and its methyl ester with ammonia and amines to form *erythro*-β-hydroxy-L-aspartic acid and N-substituted analogs is discussed and some products are described.

The three isomers of epoxysuccinic acid (*trans*-racemate and *cis-meso*) have been related to the corresponding tartaric and chloromalic acids.[1–3] The *cis-meso* form has been prepared by hydrogen peroxide oxidation of benzoquinone[4] and by nitric acid oxidation of the macrolide antibiotic, carbomycin,[5] and both the *cis* isomer and the *trans* racemate can be prepared by tungstate-catalyzed hydrogen peroxide oxidation of, respectively, maleic and fumaric acids.[6] To obtain the pure (−)-*trans* isomer, however, a fermentative preparation was used since fairly high yields had been reported from the fermentation of glucose with *Aspergillus fumigatus*.[7–10] We observed yields from this mold of over 20 g./l. of fermentation broth or a 40% molar conversion calculated from the glucose moiety of the sugar in the crude molasses used. Other molds, particularly *Penicillium viniferum* and *Monilia formosa* also produce this acid.[11–15]

The oxirane ring as substituted in epoxysuccinic acid is less reactive to acidic reagents than many epoxides,[16] permitting selective reactions at the carboxyl groups. Thus, either free epoxysuccinic acid or its slightly soluble barium salt, the form in which the acid was isolated from fermentation broths, could be esterified in alcohols with sulfuric acid catalyst. A number of the esters so prepared are listed in Table I.

When (−)-*trans*-epoxysuccinic acid was heated with hydrochloric acid in methanol, dimethyl *erythro*-chloromalate was formed. Treatment of either this ester or dimethyl epoxysuccinate with cold aqueous ammonia led to high yields of the slightly soluble (−)-*trans*-epoxysuccinamide. Similarly other amides were pre-

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(2) R. Kuhn and R. Zell, *ibid.*, **59**, 2514 (1926).
(3) R. Kuhn and T. Wagner-Jauregg, *ibid.*, **61**, 513 (1928).
(4) E. Weitz, H. Schobbert, and H. Seibert, *ibid.*, **68**, 1166 (1935).
(5) R. B. Woodward, *Angew. Chem.*, **69**, 50 (1957).
(6) G. B. Payne and P. H. Williams, *J. Org. Chem.*, **24**, 54 (1959).
(7) J. H. Birkinshaw, A. Bracken, and H. Raistrick, *Biochem. J.*, **39**, 70 (1945).
(8) J. Moyer, U. S. Patent 2,674,561, Sept. 8, 1950 (to Secretary of Department of Agriculture).
(9) W. Martin and J. Foster, *J. Bacteriol.*, **70**, 405 (1955).
(10) It is interesting that fumagillin another *Aspergillus fumigatus* metabolite, contains two epoxide groups: [J. Landquist, *J. Chem. Soc.*, 4237 (1956), and D. S. Tarbell, R. M. Carman, D. D. Chapman, K. R. Huffman, and N. J. McCorkindale, *J. Am. Chem. Soc.*, **82**, 1005 (1960)].
(11) K. Sakaguchi, T. Inoue, and Y. Tada, *J. Agr. Chem. Soc. Japan*, **13**, 241 (1937); *Proc. Imp. Acad.* (Tokyo), **13**, 9 (1947).
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(15) K. Sakaguchi and T. Inoue, *J. Agr. Chem. Soc. Japan*, **16**, 1015 (1940).
(16) For quantitative determinations of the rates of cleavage of a number of epoxysuccinic acid derivatives in pyridinium hydrobromide-acetic acid at 30° see M. W. Miller, *J. Org. Chem.*, **28**, 1148 (1963).

TABLE I

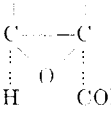
ESTERS OF (−)-*trans*-EPOXYSUCCINIC ACID

| R | M.p. (B.p.), °C. | $[\alpha]^{25}D$ Solvent[a] | Yield, % | Formula | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found |
|---|---|---|---|---|---|---|---|---|
| Methyl | m. 74–75 | −125° (Ethanol) | 96 | $C_6H_8O_5$ | 45.00 | 45.03 | 5.04 | 5.14 |
| Ethyl | b. 54–56 (0.5 mm.) | −110° (Ethanol)[b] | 32 | $C_8H_{12}O_5$ | 51.06 | 51.20 | 6.43 | 6.65 |
| Allyl | b. 78–85 (0.1 mm.) | −102° (Ethanol)[c] | 14 | $C_{10}H_{12}O_5$ | 56.60 | 56.49 | 5.70 | 5.98 |
| Propargyl | m. 70–71 | −123° (Ethanol) | 59 | $C_{10}H_8O_5$ | 57.69 | 57.83 | 3.87 | 4.05 |
| Octyl | b. 120–140 (0.05 mm.) | −58° (Methanol) | 63 | $C_{26}H_{36}O_5$ | 67.38 | 67.20 | 10.18 | 9.93 |
| Dodecyl | m. 43–44 | −27° (Dioxane) | 98 | $C_{28}H_{52}O_5$ | 71.75 | 71.51 | 11.18 | 11.39 |
| Benzyl | b. 190–194 (0.1 mm.) | −66° (Ethanol)[d] | 63 | $C_{18}H_{16}O_5$ | 69.22 | 69.23 | 5.16 | 5.44 |
| Phenyl | m. 100–102 | −144° (Ethanol) | 78 | $C_{18}H_{12}O_5$ | 67.60 | 67.11 | 4.26 | 4.39 |
| Pentachlorophenyl[e] | m. 206–207 | −68° (Chloroform) | 51 | $C_{16}H_2O_5Cl_5$ | 30.56 | 31.08 | 0.32 | 0.55 |

[a] $c$ = 1.0 in all cases. [b] $n^{25}D$ 1.4344. [c] $n^{25}D$ 1.4643. [d] $n^{25}D$ 1.5523. [e] Chlorine (%: calcd., 56.39; found, 56.24.

TABLE II

AMIDES OF (−)-*trans*-EPOXYSUCCINIC ACID



| R | M.p., °C. | Yield, % | Formula | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|
| Hydrogen[a] | 208–209 | 97 | $C_4H_6N_2O_4$ | 36.92 | 36.67 | 4.65 | 4.40 | 21.54 | 21.57 |
| Methyl[b] | 238–242 | 25 | $C_6H_{10}N_2O_3$ | 45.56 | 45.32 | 6.37 | 6.31 | 17.71 | 17.92 |
| Allyl[c] | 209–210 | 40 | $C_{10}H_{14}N_2O_3$ | 57.13 | 56.94 | 6.71 | 6.61 | 13.33 | 13.09 |
| Benzyl[d] | 177–178 | 46 | $C_{18}H_{18}N_2O_3$ | 69.66 | 69.54 | 5.85 | 5.81 | 9.03 | 9.02 |
| Octyl | 168–169.5 | 97 | $C_{20}H_{42}N_2O_4$ | 67.75 | 67.66 | 10.80 | 10.36 | 7.90 | 8.13 |
| Nonyl | 169–171 | 96 | $C_{22}H_{42}N_2O_3$ | 67.06 | 68.85 | 11.07 | 10.97 | 7.32 | 7.40 |
| Decyl | 150–153 | 83 | $C_{24}H_{46}N_2O_3$ | 70.19 | 70.00 | 11.29 | 11.24 | 6.82 | 7.48 |
| Undecyl | 162–164 | 85 | $C_{26}H_{50}N_2O_3$ | 71.18 | 70.94 | 11.49 | 11.48 | 6.39 | 6.45 |
| Dodecyl | 162–164 | 89 | $C_{28}H_{54}N_2O_3$ | | | | | | |
| Hexadecyl | 143–144 | 86 | $C_{36}H_{70}N_2O_3$ | 74.68 | 74.12 | 12.19 | 12.11 | 4.84 | 5.34 |
| Octadecyl | 146–147 | 87 | $C_{40}H_{78}N_2O_3$ | | | | | 4.53 | 4.50 |
| β-Hydroxyethyl | 156–160 | 91 | $C_8H_{14}N_2O_5$ | 44.03 | 43.96 | 6.47 | 6.45 | 12.84 | 12.76 |
| Phenyl | 221–224 | 77 | $C_{16}H_{14}N_2O_4$ | 68.07 | 68.25 | 5.00 | 5.13 | 9.92 | 9.83 |
| Amino ($NH_2$)[e] | 138 dec. | 68 | $C_4H_8N_4O_5$ | 30.00 | 29.44 | 5.04 | 4.84 | 34.99 | 34.86 |
| Anisyl | 256–258 | 75 | $C_{18}H_{18}N_2O_5$ | 63.15 | 63.41 | 5.30 | 5.30 | | |

[a] $[\alpha]^{25}D$ −68° (*c* 1.0 in water). [b] $[\alpha]^{25}D$ −92° (*c* 1.0 in ethanol). [c] $[\alpha]^{25}D$ −93° (*c* 1.0 in pyridine). [d] $[\alpha]^{25}D$ −71° (*c* 1.0 in pyridine). [e] $[\alpha]^{25}D$ −97° (*c* 1.0 in ethanol).

pared by interaction of dimethyl epoxysuccinate with two moles of a primary amine in suitable solvents (Table II).

The aromatic esters and amides shown in the tables were prepared by way of (−)-*trans*-epoxysuccinyl chloride which was obtained by treatment of (−)-*trans*-epoxysuccinic acid with phosphorus pentachloride and rapid distillation in a twin-bulb flask.

Although inert in many acidic circumstances, the oxirane ring of epoxysuccinic acid was opened rapidly in aqueous alkali. When (−)-*trans*-epoxysuccinic acid was warmed with aqueous ammonia, for example, β-hydroxyaspartic acid formed. Of the four possible isomers of this amino acid (racemates of the *erythro* and of the *threo* forms) an *erythro* isomer was considered probable since amines are known generally to open uncomplicated epoxide rings by a simple Sn2 mechanism with inversion of configuration. Our isomer has essentially the same specific rotation ($[\alpha]^{25}D$ +49°, *c* 0.143 *N* in hydrochloric acid) as the *erythro*-β-hydroxy-L-aspartic acid prepared recently by transamination between glutamate and oxaloglycolate ($[\alpha]^{25}D$ +51° ± 2°, *c* 1.59 in *N* hydrochloric acid.)[17] It also seems to correspond to the "*d-anti*-hydroxyaspartic acid" prepared long ago[18] by heating chloromalic acid with ammonia, a method not entirely stereospecific.[19] While other syntheses have been reported lately,[20–22] these also have been biochemical in nature or involve isomer separation, and the stereospecific route from (−)-*trans*-epoxysuccinic acid is the method of choice for preparing pure *erythro*-β-hydroxy-L-aspartic acid.

Pancreatic digests of casein contain *erythro*-β-hy-

(17) H. J. Sallach, *J. Biol. Chem.*, **229**, 437 (1957).

(18) H. D. Dakin, *ibid.*, **48**, 273 (1921).

(19) M. L. Kornguth and H. J. Sallach, *Arch. Biochem. Biophys.*, **91**, 39 (1960).

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(22) D. E. Metzler, J. B. Longenecker, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 639 (1954).

TABLE III

N,N′,N″-TRIALKYL-β-HYDROXYASPARTAMIDES, RNHCOCHOHCH(NHR)CONHR

| R | M.p., °C | Yield, % | Formula | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|
| Methyl[a] | 125–126 | 98 | $C_7H_{15}N_3O_3$ | 44.43 | 44.10 | 7.99 | 7.75 | 22.21 | 21.67 |
| Allyl | 136–137 | 80 | $C_{13}H_{21}N_3O_3$ | 58.41 | 58.37 | 7.92 | 8.17 | 15.72 | 16.07 |
| Octyl | 120–122 | 60 | $C_{28}H_{57}N_3O_3$ | 69.51 | 69.50 | 11.88 | 12.03 | 8.69 | 8.69 |
| Decyl | 119.5–120.5 | 56 | $C_{34}H_{69}N_3O_3$ | 71.90 | 71.52 | 12.25 | 11.81 | ... | ... |
| Octadecyl | 111–112 | 97 | $C_{58}H_{117}N_3O_3$ | ... | ... | ... | ... | 4.73 | 4.75 |
| Phenyl | 234–237.5 (s-230) | 83 | $C_{22}H_{21}N_3O_3$ | 70.38 | 70.64 | 5.64 | 5.53 | 11.19 | 11.31 |

[a] $[\alpha]^{25}D$ +60° (*c* 1.0 in ethanol).

droxy-L-aspartic acid,[23] and in several biological systems studied it is the active diastereoisomer.[19] In studies on nitrogen fixation a hydroxyaspartic acid, isomer unspecified, was isolated from cultures of azotobacter cells grown with elemental nitrogen as the sole nitrogen source.[24] The *Amanita phalloides* toxin, phallacidin, is distinguished from the related phalloidin by the presence of *erythro*-β-hydroxy-D-aspartic acid as a component of the cyclic polypeptide structure instead of D-threonine.[25,26] Hydroxyaspartic acid has been shown to cause bacteria to mutate,[27] and it inhibits competitively the enzyme aspartate aminopherase.[20]

Although good yields of diamides were obtained by controlled addition of two moles of amine to dimethyl epoxysuccinate, three or more moles of a primary amine reacted with epoxysuccinic esters to form N-substituted β-hydroxyaspartic amides (Table III).

The triphenyl analog was prepared by heating a solution of epoxysuccinic acid in aniline to reflux for a short period. Treatment of N,N′,N″-triphenyl-β-hydroxyaspartamide with methanolic hydrogen chloride yielded a half methyl ester, $C_6H_5NHCOCH(NHC_6H_5)$-$CHOHCOOCH_3$, the amine salt inhibiting esterification at the proximate amide group.

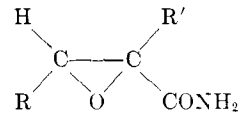
Although epoxysuccinic acid is a much stronger acid than succinic acid (epoxysuccinic acid $K_1^{19} = 1.2 \times 10^{-2}$, succinic acid $K_1^{25} = 6.4 \times 10^{-5}$) it is not very toxic. Selected toxicity data are shown in Table IV.

TABLE IV

SELECTED TOXICITIES

| Compound | Toxicity (acute oral $LD_{50}$) |
|---|---|
| (−)-*trans*-Epoxysuccinic acid | >2.0 g./kg. (mouse) |
| Dimethyl (−)-*trans*-epoxysuccinate | >2.5 g./kg. (mouse) |
| N,N′-Bis-*n*-decylepoxysuccinamide | >3.0 g./kg. (mouse) |
| N,N′,N″-Tridecyl-β-hydroxyaspartamide | >3.0 g./kg. (mouse) |
| (−)-*trans*-Epoxysuccinonitrile | 0.025 g./kg. (rat) |

Mild hypnotic activity has been reported for dialkylglycidamides of the type shown below.[28] Several epoxysuccinamides also showed hypnotic effects, the



octyl derivative, for example, causing a mild decrease in motor activity with a duration of 90 minutes in mice.

The novel structure (−)-*trans*-epoxysuccinonitrile was prepared in about 50% yield by carefully controlled heating of (−)-*trans*-epoxysuccinamide with phosphorus oxychloride. It was an effective miticide, 0.35% emulsions giving effective control of *Tetranychus atlanticus*. N,N′-Bis-*n*-decylepoxysuccinamide was a specific insecticide for the pea aphid, an economic pest on several legume crops. Propargyl (−)-*trans*-epoxysuccinate showed notable antifungal activity with minimum inhibitory concentrations at 100 μg./ml. against *Trichophyton sulfuricum*, *Phiolophora verrucosa*, the *Aspergillus flavus-oryzae* group, *Pullularia pullulans* and *Cladosporium herbarium*. *erythro*-β-Hydroxy-L-aspartic acid was active against Gram-negative bacteria on Witkin synthetic medium, some examples of minimum inhibitory concentrations (μg./ml.) being: *Escherichia coli* 6.25, *Aerobacter aerogenes* 6.25, *Proteus vulgaris*, 1.56, *Pseudomonas aeruginosa* 6.25.

## Experimental[29]

Only a few typical or key experiments are reported, most of the esters and amides having been prepared by conventional techniques. All preparations involving amines were carried out in a $CO_2$-free nitrogen atmosphere.

The production of (−)-*trans*-epoxysuccinic acid by an improved strain of *Aspergillus fumigatus* Fres. was accomplished using media based on those described in earlier publications (*e.g.*,[8,16]).

**Isolation of Barium Epoxysuccinate.**—A 130 gal. batch of fermentation whole broth was adjusted to a pH of 1.5 with concd. hydrochloric acid, 4.56 kg. of cellulose filter aid (Super-Cel) added, and the mixture filtered through a small 12-plate filter-press. To the filtrate, with stirring, was added 10.94 kg. of barium carbonate. The pH was adjusted to 7 with sodium hydroxide pellets, and stirring continued for 10 hr. In this way 13.82 kg. (dry wt.) of barium epoxysuccinate was obtained. Barium analysis (as $BaSO_4$): calcd., 51.4; found, 51.2%.

**Conversion of Barium (−)-*trans*-Epoxysuccinate to (−)-*trans*-Epoxysuccinic Acid.**—A 100 g. sample of barium epoxysuccinate, assaying 85% purity, was suspended in 100 ml. of dry tetrahydrofuran and the temperature maintained at 5–10° while 18.5 ml. of concd. sulfuric acid was added dropwise with stirring during 2.5 hr. The barium sulfate was removed, washed with fresh cold solvent, and the filtrate evaporated to give 40 g. of dry, tan crystalline epoxysuccinic acid, assaying 95% purity (95% yield), as well as 5.25 g. of a gummy residue. One recrystallization from dry dioxane gave nearly pure white crystals with little loss, m.p. 182–184°. When acetone was used as the acidification solvent,

(23) H. J. Sallach and M. L. Kornguth, *Biochim. Biophys. Acta*, **34**, 582 (1959).

(24) A. I. Virtanen and N. Saris, *Suomen Kem.*, **30B**, 100 (1957).

(25) T. Wieland and H. W. Schnabel, *Ann.*, **657**, 218 (1962).

(26) The antibiotic telomycin has been shown to contain the amino acid *erythro*-β-hydroxy-L-leucine [J. C. Sheehan, K. Maeda, A. K. Sen, and J. A. Stock, *J. Am. Chem. Soc.*, **84**, 1303 (1962)].

(27) J. B. Clark, *Proc. Okla. Acad. Sci.*, **34**, 114 (1953); *Chem. Abstr.*, **49**, 8373f (1955).

(28) R. S. Shelton and K. W. Wheeler, U. S. Patent 2,493,090 (1950); *Chem. Abstr.*, **44**, 2552 (1950).

(29) Melting points were determined on a calibrated Kofler micro hot stage.

a 66% yield was obtained. When water was used, the yield was 60%.

**Dimethyl (−)-*trans*-Epoxysuccinate from Barium (−)-*trans*-Epoxysuccinate.**—A 2000 g. sample of 85% pure barium epoxysuccinate (6.36 moles) was suspended in 6 l. (149 moles) of dry methanol. The mixture was cooled to 10–15° and 452 ml. (832 g., 8.49 moles) of concd. sulfuric acid added slowly with stirring during 4 hr. After standing overnight 240 g. (2 moles) of anhydrous magnesium sulfate was added and the mixture refluxed with stirring for 5 hr. Upon addition of 187 g. (1.87 moles) of powdered calcium carbonate the temperature was raised to 60°, the methanol solution filtered through a heated funnel, and the solvent removed at reduced pressure. In this way 820 g. (5.12 moles) of ester was obtained as a first crop and 92 g. by sublimation or recrystallization of the residue for a 96% yield of nearly pure white dimethyl epoxysuccinate, m.p. 72–75°. Sublimation at 50–80° (2 mm.) yielded large colorless needles, m.p. 74–75°.

Methyl (−)-*trans*-epoxysuccinate also was prepared in 70–75% yields by Fischer esterification of the free acid. By similar methods (from the barium salt) or by use of the conventional Fischer esterification technique with separation of water (from epoxysuccinic acid) the other esters shown in Table I were prepared.

**Conversion of Barium (−)-*trans*-Epoxysuccinate to Dimethyl *erythro*-Chloromalate.**—A 100 g. sample of 85% pure barium epoxysuccinate (0.32 mole) was added to a solution of 58.5 g. (1.6 moles) of hydrogen chloride in dry methanol. The mixture was stirred at about 25° for 4 hr., allowed to stand overnight, then refluxed with stirring for 4 hr. After filtration the methanol was evaporated and the residual oil dissolved in ether. A heavy brown ether-insoluble liquid which separated was discarded, and the ether solution washed with bicarbonate solution, dried, and freed of solvent. The remaining oil, 41.6 g. (67% yield) was fractionally distilled to give 35.6 g. of colorless dimethyl (−)-chloromalate, b.p. 94–96° (0.8–1.0 mm.) $n^{25}D$ 1.4585, $d^{25}$ 1.3536, $[\alpha]^{25}D$ −37° (neat); reported for dimethyl (−)-chloromalate[32]: $n^{20}D$ 1.4594, $d^{20}$ 1.3598, $[\alpha]^{22}D$ −22° (neat).

*Anal.* Calcd. for $C_6H_9ClO_5$: C, 36.66; H, 4.61; Cl, 18.04. Found: C, 36.67; H, 4.68; Cl, 17.71.

When 30 g. of (−)-*trans*-epoxysuccinic acid was used as the starting material, 45.7 g. of crude oil and 30.4 g. (71% yield) of colorless product, $n^{25}D$ 1.4580, was obtained.

**(−)-*trans*-Epoxysuccinamide (a) from Dimethyl (−)-*trans*-Epoxysuccinate.**—A 70.0 g. (0.437 mole) sample of dimethyl epoxysuccinate was stirred with 200 ml. of cold ammonium hydroxide (29% ammonia), using a strong stirrer in an open beaker for 10–15 min. The ester dissolved as the amide separated, and the product was removed by filtration and washed with a little cold water. The crude dry epoxysuccinamide weighed 60 g., m.p. 203–206°. Recrystallization from water gave 52 g. (97% yield) of pure material, m.p. 205–208° dec.

**(b) From Methyl *erythro*-Chloromalate.**—A 23.0 g. (0.119 mole) sample of dimethyl chloromalate was added to 100 ml. of concd. (29% ammonia) ammonium hydroxide at 0–5° with stirring. The solution became red, and after 45 min. a fine precipitate was present. Filtration yielded 11.27 g. (73% yield) of dry, crude epoxysuccinamide, m.p. 190–200°. Recrystallization from water gave material of m.p. 205–208°, and the m.p. of a mixture with the product from (a) above was the same.

The other amides shown in Table II were prepared by gradual addition of two moles of amine to dimethyl epoxysuccinate in a suitable solvent (*e.g.*, methanol) with heating and stirring.

**(−)-*trans*-Epoxysuccinonitrile from (−)-*trans*-Epoxysuccinamide.**—A stirred mixture of 5.4 g. (0.0415 mole) of dry epoxysuccinamide and 30 ml. of phosphorus oxychloride was heated rapidly under dry nitrogen. During 35 min. the amide dissolved as the temperature of the oil-bath was raised from 80–100°, and 5 min. later heating was discontinued. After cooling to room temperature excess phosphorus oxychloride was removed by distillation at 10 mm. pressure, and epoxysuccinonitrile separated as the concentration progressed. The crude product was removed by filtration, sucked very dry (otherwise there were fume-offs), washed twice with ice water, and purified by sublimation at 85° (0.1 mm.). The purified substance, 1.9 g. (49% yield) formed thick white prisms, m.p. 130–130.5°, $[\alpha]^{25}D$ −71° (*c* 1.0, ethanol).

*Anal.* Calcd. for $C_4H_2N_2O$: C, 51.06; H, 2.14; N, 29.78. Found: C, 51.10; H, 1.92; N, 29.29.

Many other techniques of nitrile formation failed when applied to (−)-*trans*-epoxysuccinamide. Purification also was accomplished by recrystallization of 6 g. of crude product from 35 ml. of boiling 95% ethanol.

**(−)-*trans*-Epoxysuccinyl Chloride.**—This compound was prepared by heating (−)-*trans*-epoxysuccinic acid with phosphorus pentachloride, a method described earlier for the *cis meso*-isomer.[30,31] Yields were erratic, varying from 50–90%. One of the better runs is described below: A 182 g. (0.875 mole) sample of fresh, finely divided phosphorus pentachloride (Mallinckrodt Analytical Reagent grade) was weighed rapidly and transferred to a 1 l. round-bottom flask fitted with an Allihn condenser (protected by a Drierite tube) and provided with an oil-bath heater. Then 55 g. (0.417 mole) of dry, pulverized epoxysuccinic acid was added rapidly. Rapid addition is required because if reagents are good reaction may set in at once. As soon as foaming abated the mixture was heated for 20 min. at 85°. The cooled mixture was transferred to a twin-bulb flask, and most of the phosphorus oxychloride (92 g., 0.600 mole) removed at 34 mm. pressure by heating with a flame. After removal of the forerun 80.3 g. of colorless liquid which partially crystallized was collected at 2–5 mm. pressure. Redistillation in the same apparatus yielded, after a small forerun, 63.5 g. (90%) of a colorless product which crystallized on storage overnight in the refrigerator. After several days in the refrigerator some sublimation had occurred. The product melted at 42–45°. Sometimes, when the product failed to crystallize, extraction or trituration of the liquid with hexane purified it enough so that it would crystallize.

***erythro*-β-Hydroxy-L-aspartic Acid.**—A 10 g. sample of 95% pure (−)-*trans*-epoxysuccinic acid (0.0719 mole) was dissolved in 200 ml. of 28–30% ammonium hydroxide solution with cooling. The solution was warmed on the steam bath (hood) for 2 hr. at a temperature as high as the evolution of ammonia gas permitted (42°). The solvent then was removed at reduced pressure at a temperature below 40°. The residual red sirup was diluted with (twice its volume of water and acidified to pH 3.0 with 12% hydrochloric acid. The product crystallized, and was separated by filtration. The weight of the first crop was 5.95 g., and concentration of the filtrate gave a second crude crop of 8.20 g. Recrystallization from water yielded 10.1 g. of pure material, which decomposed above 200° without a definite m.p.

*Anal.* Calcd. for $C_4H_7NO_5$: C, 32.33; H, 4.73; N, 9.39. Found: C, 32.50; H, 4.85; N, 8.98; $[\alpha]^{25}D$ +49° (*c* 0.143, 1 *N* hydrochloric acid).

**N,N′,N″-Triphenyl-β-hydroxyaspartamide.**—A mixture of 180 g. (1.36 moles) of (−)-*trans*-epoxysuccinic acid and 1800 ml. (19.37 moles) of aniline was stirred and heated under carbon dioxide-free nitrogen. When the temperature reached 120°, all the acid had dissolved, and water was evolved. The temperature was raised to 145° by distilling 150 ml. of aniline-water mixture. After 3 hr. the mixture, while still hot, was poured into a beaker. After cooling, the nearly solid mixture was filtered, the residue, which retained much solvent, was washed with benzene, then recrystallized from pyridine and from acetonitrile. The product, freed of solvent, weighed 233 g., m.p. 233–236° (s. 210°). A second crop was obtained from the benzene, pyridine and acetonitrile solutions. It weighed 192 g. m.p. 225–232° (s. 208°). The total yield, then, was 425 g. (83%). A sample of first crop material, purified by a second recrystallization from acetonitrile, melted from 234–237.5° (droplets on cover-glass from 230°).

**Methyl 2-Oxy-3-phenylaminosuccinate-4-N-phenylcarboxamide.**—A 4.05 g. (0.0166 mole) sample of N,N′,N″-triphenyl-β-hydroxyaspartamide was dissolved in methanol saturated with hydrogen chloride and the solution stored at room temperature for 8 days. A slight flocculent precipitate was removed by filtration and all volatile material removed at aspirator pressure. The residue was dissolved in ether and the ether solution washed several times with 5% sodium bicarbonate solution. The solvent was removed at reduced pressure to yield 3.0 g. of crude product, m.p. 120–124°. Three recrystallizations from hexane gave colorless crystals of the half-ester, m.p. 126–128°.

*Anal.* Calcd. for $C_{17}H_{18}N_2O_4$: C, 64.95; H, 5.77; N, 8.91. Found: C, 64.69; H, 5.78; N, 8.85.

**N,N′,N″-Trioctyl-β-hydroxyaspartamide.**—A mixture of 20 g. (0.125 mole) of dimethyl epoxysuccinate, 48.4 g. (62.4 ml., 0.374 mole) of *n*-octylamine, and 200 ml. of dry ethanol was refluxed for 24 hr. The mixture became nearly solid during the last 2 hr., and on cooling solidified. The entire contents of the flask (hard soap-like consistency) was dried to constant weight at 0.5 mm. pressure. The crude product weighed 35.8 g., m.p. 115–120°.

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This material gelled in most organic solvents, but was recrystallized from about 5 l. of dioxane 3 times and once from 3.5 l. of chloroform to obtain white waxy crystals, m.p. 120–122°. Other close analogs showed the same tendency to gel in most organic solvents at conventional concentrations.

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# Some Aspects of the Fate and Relationship of the N-Methyl Group of Morphine to its Pharmacological Activity[1a]

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The chemical synthesis of N-trideuteriomethylnormorphine is described. Pharmacologically it is less potent than morphine although its duration of action is unaffected. Compared with morphine its N-demethylation by rat liver microsomal enzymes occurs less readily and requires a larger energy of activation. A larger $K_m$ indicates that it is less strongly bound to the enzyme. The $V_m$'s of rat liver N-demethylating enzymes were found to be inversely proportional to the biological potencies of the drugs, whereas the $K_m$ increases as the potency increases. Diminished capacity of the enzymes of tolerant rats to N-demethylate morphine was shown to be due to reduced availability of enzymes rather than alteration in enzyme structure. Both the *l*- and *d*-isomers of analgesics apparently are demethylated by the same microsomal enzymes. The enzymes from male and female rats are probably identical. By two separate methods it was shown that nalorphine inhibited the N-demethylation of morphine noncompetitively. The results obtained with rat liver microsomal enzymes fail to support either Beckett's theory on the mechanism of analgesia or Axelrod's theory on the development of tolerance to some of the actions of these drugs. It is concluded that these enzymes are not suitable as models by which these theories may be judged.

The mechanism by which narcotic agents produce analgesia is not known nor has any satisfactory mechanistic explanation been presented for the development of tolerance to some of their actions. Recently, two theories have been advanced which have implied that N-dealkylation of these agents is essential to these phenomena.

Beckett, *et al.*,[2] believe that not the mere presence of these drugs at the receptors in the brain, but the subsequent N-demethylation which occurs there constitutes the first step in the reaction sequence that leads to analgesia. Nalorphine is thought to antagonize the actions of narcotic analgesics by virtue of a greater affinity for the receptor sites plus a much slower N-dealkylation therein.

Axelrod was prompted to study the effects of tolerance to narcotic analgesics on the capacities of liver microsomal enzymes from rats to demethylate these drugs by the finding that the enzymes are capable of N-dealkylating various drugs.[3,4] He found considerable reduction in the capacities of the livers from tolerant rats to N-demethylate morphine and other analgesics which exhibit cross tolerance to morphine.[5] When nalorphine was administered with morphine, the diminution in enzymic activity of the liver was significantly less than when the tolerant rats received morphine alone. Axelrod suggested that the continual interaction of these agents with the enzymes that N-dealkylate them inactivates the enzymes. Similarly he inferred that the continual interaction of these drugs with their receptors in the central nervous system may inactivate the receptors. In other words, if the liver microsomal enzymes are used as models for the receptors in the brain it follows that tolerance may occur as a result of unavailability of receptor sites.

Substitution of N-trideuteriomethylmorphine for morphine in *in vivo* and *in vitro* studies should provide a direct test of the theory of Beckett, *et al.*,[6] on the nature of binding to the central nervous system receptors as well as on the mechanism of narcotic analgesia. The two compounds differ with respect to the zero-point energies of the C–H and C–D bonds as well as the masses of the two methyl groups, but should combine with identical receptors both in the central nervous system and the N-demethylating enzymes. Since the theory is based on an inter-related consideration of stereochemical configuration, physicochemical properties, and the enzymatic N-dealkylation, comparisons of the *in vivo* potencies, the rates of enzymatic N-demethylation, the energies of activation for N-demethylation, and the Michaelis constants of the N-demethylating enzymes for these substrates, have been made to obtain information for judging the theory. Since the degree of ionization of the basic groups is

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