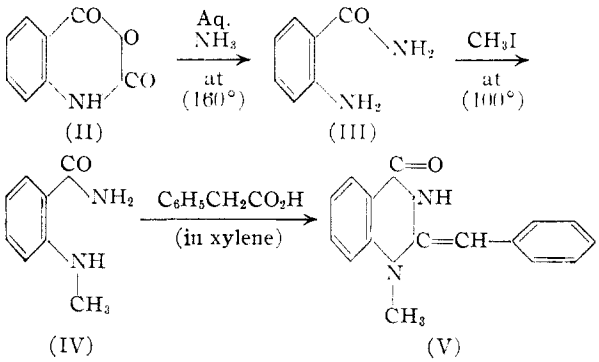
The structure (I) for glycosin has been confirmed by synthesis from isatoic anhydride (II), m.p. 240°, which on treatment with aqueous ammonia (30%) at 160° gave anthranilide (III), m.p. 108° (yield, 90%). The latter on heating with methyl iodide at 100° for 6 hours in a sealed tube gave N-methylanthranilamide (IV), m.p. 159° (yield, 90%). N-Methylanthranilamide when refluxed with a molar proportion of phenylacetic acid in xylene (dried over sodium) with excess of phosphorus pentoxide for one hour produced glycosin, $C_{16}H_{14}N_2O$, m.p. 155° (I) (yield, 55% of the theoretical). *Anal.* Calcd. for $C_{16}H_{14}N_2O$: C, 76.80; H, 5.60; N, 11.20; NMe, 6.00; M.W., 250. Found: C, 76.45; H, 5.45; N, 11.34; NMe, 6.24; M.W., 244 by Rast and 247 by chloroplatinate method.



(II) (III) (IV) (V)

Synthetic and natural glycosin showed no depression in their mixed melting points and also in the mixed melting points of their salts. On ozonolysis and on oxidation with periodic acid synthetic glycosin produces benzaldehyde like the natural product and their infrared absorption spectra are exactly identical. Synthesis of glycosin, its ozonolysis and periodic acid oxidation experiments have enabled the authors to settle the molecular formula of glycosin as $C_{16}H_{14}N_2O$ and not $C_{15}H_{12}N_2O$, a point which cannot be decided from the analysis of the base and its salts.[1]

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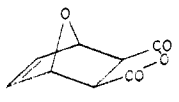
RECEIVED JULY 22, 1953

CIS-ADDITION IN THE BROMINATION OF A BICYCLIC OLEFIN

Sir:

Reaction of *exo-cis*-3,6-endoxo-Δ^4-tetrahydrophthalic anhydride (I) with bromine in oxygen-free methylene chloride yields two saturated dibromides: IIA (55%), m.p. 163°; *Anal.* Calcd. for $C_8H_6O_4Br_2$: C, 29.48; H, 1.86; Br, 49.04. Found: C, 29.56; H, 1.96; and IIB (36%), m.p. 331°; *Anal.* Found: C, 29.55; H, 1.99; Br, 48.99.



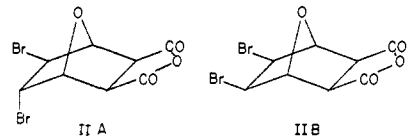
I

Each was degraded *via* the sequence anhydride → amidic acid → imide. IIA gave an imide (IIIA), m.p. 221° (d.); *Anal.* Calcd. for $C_8H_7O_3NBr_2$: C, 29.56; H, 2.17; N, 4.31. Found: C, 29.72; H, 2.07; N, 4.17. IIB gave an imide (IIIB), m.p. 297° (d.); *Anal.* Found: C, 29.85; H, 2.14; N, 4.28. Either IIIA or IIIB with zinc in acetic acid gave the known[1] *exo-cis*-3,6-endoxo-Δ^4-tetrahydrophthalimide (IV). The *endo*-isomer[1] (V) of IV is stable under the debromination conditions.

Partial optical resolution of the acid (VI) of IIA *via* the quinine salt gave (−)-VI, $[\alpha]_D$ −77.5°; *Anal.* Calcd. for $C_8H_8O_5Br_2$: C, 27.93; H, 2.34. Found: C, 28.04; H, 2.07. The infrared spectra of (−)-IIA, $[\alpha]_D$ −56°, and of (−)-dimethyl ester of VI, $[\alpha]_D$ −73°, were identical with those of the corresponding racemates.

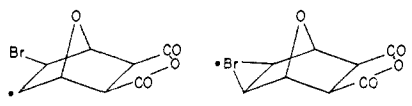
Partial optical resolution of the half-methyl ester (VII) prepared from IIB gave (+)-VII $[\alpha]_D$ +2.5°, and (−)-VII ,$[\alpha]_D$ −3.3°; *Anal.* Calcd. for $C_9H_{10}O_5Br_2$: C, 30.19; H, 2.81. Found: C, 30.41; H, 3.16, infrared spectrum identical with that of racemic VII. Methylation of (+)-VII or (−)-VII gave *optically inactive* dimethyl ester, m.p. 200°, alone or mixed with a sample prepared from IIB; *Anal.* Calcd. for $C_{10}H_{12}O_5Br_2$: C, 32.28; H, 3.25. Found: C, 31.92; H, 3.29. Hydrolysis of (+)-VII gave optically inactive acid (VIII) m.p. 331°; *Anal.* Found: C, 28.18; H, 2.39.

IIA is racemic and therefore has the *trans*-dibromide configuration. IIB is a *meso* compound and therefore has the *cis*-dibromide configuration. By application of the *exo*-addition rule,[2] the *exo*-configuration is assigned to the bromines of IIB.



IIA IIB

Formation of IIB appears to occur largely by a free radical reaction. In darkness or in polar solvents (acetic acid, ethyl acetate), bromination of I gives *ca.* 90% of IIA and 0% of IIB. The repulsive non-bonded interaction[3] of the "eclipsed" bromines of IIB should result in a higher activation energy[4] for formation of IIB than for IIA in a mechanism involving a random attack on a radical intermediate such as IX. Also, attack on a cyclic radical[5] (X) would lead largely to IIA.

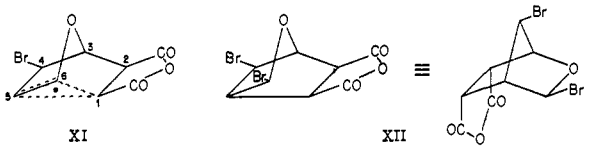


IX X

The occurrence of a high proportion of *cis*-bromination despite these considerations implies the operation of some powerful stereo-electronic demand. This may be attributable to the intermediate formation of (i) the bridged-radical (XI) re-

(1) H. Kwart and I. Burchuk, THIS JOURNAL, **74**, 3094 (1952).
(2) K. Alder and G. Stein, *Ann.*, **515**, 185 (1935).
(3) O. Bastiansen and O. Hassel, *Tids. Kjemi, Bergvesen Met.*, **6**, 96 (1946); O. Hassel and B. Ottar, *Acta Chem. Scand.*, **1**, 929 (1947).
(4) M. G. Evans and M. Polanyi, *Trans. Faraday Soc.*, **34**, 11 (1938).
(5) H. L. Goering, P. I. Abell and B. F. Aycock, THIS JOURNAL, **74**, 3588 (1952).

quiring stereospecific approach of bromine to C_5 from the *exo*-direction, and or (II) the unstable α-bromoether (XII), ionic rearrangement of which to IIB is in precise steric and electronic analogy to the change camphene hydrochloride → isobornyl chloride.[6]



XI XII

The formation of *cis*-dibromide by either or both of these paths appears to be unique in the literature.

(6) (a) H. Meerwein and K. van Emster, *Ber.*, **53**, 1815 (1920); **55**, 2500 (1922); (b) P. D. Bartlett and I. Pöckel, THIS JOURNAL, **59**, 820 (1937); **60**, 1585 (1938); (c) T. P. Nevell, E. de Salas and C. L. Wilson, *J. Chem. Soc.*, 1188 (1939).

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RECEIVED JULY 1, 1953

PARTICIPATION OF ATP AND COENZYME A IN THE ENZYMATIC DECARBOXYLATION OF MALONIC ACID[1]

Sir:

Malonic acid was previously shown to be an intermediate metabolite of uracil degradation by bacterial enzymes.[2,3] More recently decarboxylation of malonic acid was observed with dried bacterial cells and crude extracts.[4] It has now been found that the enzymatic decarboxylation of malonic acid requires adenosinetriphosphate (ATP) and coenzyme A (CoA) and an activated form of malonate is proposed as an intermediate.

Pseudomonas fluorescens strain TR-23,[5] a strictly aerobic microörganism, was grown for about 20 hours at 26°, with constant shaking, in a medium containing 1% NH_4Cl, 0.5% disodium malonate, 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.1% Difco yeast extract. Cell-free extracts were prepared by grinding the washed cells with alumina (Alcoa A-301) in the presence of reduced glutathione (1.5 mg. of the sodium salt per g. of wet cells), extracting with 6 parts of 0.02 *M* phosphate buffer (*p*H 7.0), and centrifuging at 25,000 × *g* for 30 minutes.

A reaction mixture (2.0 ml.) containing 0.1 ml. of the crude extract (1.43 mg. protein), 100 μM. KF, 20 μM. reduced glutathione (sodium salt), 10 μM. $MgCl_2$, 200 μM. sodium acetate buffer (*p*H 5.8), 100 units CoA, 10 μM. ATP (sodium salt), 50 γ cocarboxylase and 100 μM. sodium malonate was incubated under pure nitrogen at 30° for 30 minutes. In the complete system 28.8 μM. of carbon dioxide was evolved. When ATP and CoA were omitted, only 1.9 μM. of carbon dioxide was produced. Pretreatment of the extracts with both Dowex-1[6] and charcoal[7] caused a more pronounced difference. With 0.1 ml. of the treated extract (0.86 mg. protein) the complete system yielded 13.5 μM. of carbon dioxide, whereas when either CoA, or ATP or both were omitted, 2.6, 0.9 and 0.2 μM. of carbon dioxide was produced, respectively. Neither Mg^{++} nor cocarboxylase affected the rate of the reaction under these conditions. There was no carbon dioxide production when malonate was omitted or the extract was treated at 100° for 5 minutes.

A reaction mixture (prepared as described above but with tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.0, instead of acetate buffer) containing 1.0 ml. of the crude extract and 200 μM. of hydroxylamine, yielded 7.4 μM. of hydroxamic acid derivatives[8] in the presence of ATP and CoA, whereas only 0.15 μM. was formed in the absence of the added cofactors. These hydrosamic acid derivatives were tentatively identified by paper chromatography (Whatman No. 3 with water-saturated butanol as solvent[9]) as (1) acethydroxamic acid (R_f: 0.51–0.53) and as (2) malonmonohydroxamic acid[10] (R_f: 0.36–0.38).

Thus the mechanism of malonate decarboxylation appears to involve activation of malonate (probably as malonyl CoA) as a primary step, analogous to the mechanism of succinate decarboxylation recently proposed for anaerobic microörganisms.[11,12] It has not yet been established whether the decarboxylation occurs at the activated carboxyl group to form an active one carbon compound and free acetate or whether the other carboxyl group is decarboxylated to produce active acetate and carbon dioxide. Since crude extracts were found to form hydroxamic acid derivatives from acetate, propionate, and succinate under the conditions described above, purification of the enzymes involved appears to be necessary to elucidate this point.

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RECEIVED JUNE 16, 1953

(1) This investigation was supported in part by a research grant (G3727) from the National Institutes of Health, Department of Health, Education and Welfare.

(2) O. Hayaishi and A. Kornberg, *J. Biol. Chem.*, **197**, 717 (1952)
(3) F. J. S. Lara, *J. Bact.*, **64**, 279 (1952).
(4) C. T. Gray, *ibid.*, **63**, 813 (1952).
(5) O. Hayaishi and R. Y. Stanier, *J. Biol. Chem.*, **195**, 735 (1952).
(6) H. Chantrenne and F. Lipmann, *J. Biol. Chem.*, **187**, 757 (1950).
(7) R. K. Crane and F. Lipmann, *ibid.*, **201**, 235 (1953).
(8) F. Lipmann and L. C. Tuttle, *ibid.*, **159**, 21 (1945).
(9) Incubation mixtures were treated with Dowex 50 (H^+ form) and then treated according to E. R. Stadtman and H. A. Barker, *J. Biol. Chem.*, **184**, 769 (1950).
(10) The author is indebted to Dr. David Lipkin for suggestions in preparing synthetic malonmonohydroxamic acid.
(11) E. A. Delwiche, E. F. Phares and S. F. Carson, *Federation Proc.*, **12**, 194 (1953).
(12) H. R. Whitley, THIS JOURNAL, **75**, 1518 (1953).
(13) The excellent technical assistance of Mrs. Natalie A. Fraser is gratefully acknowledged.

A MODEL FOR THE CONFIGURATION OF SULFHYDRYL GROUPS IN PROTEINS

Sir:

The differing reactivity of protein –SH groups and, especially, the marked increase in their reactivity upon denaturation of the protein, has been the subject of much speculation. We wish to report two sets of observations which suggest an explanation for this phenomenon.

In the first series of experiments three cysteine-