the addition of 2 *N* potassium hydroxide. After removal of a small amount of an amorphous precipitate by filtration, 10% barium chloride solution (147 cc.) was added, and the resulting suspension of precipitated barium salts was heated on the steam-bath for 30 minutes. The precipitate was collected after cooling to room temperature, washed with water, and dried (9.67 g., 56.9% Ba).

The barium salts were dissolved in hot 3 *N* hydrochloric acid (100 cc.) and decomposed by the addition of the calculated amount of 3 *N* sulfuric acid (27.8 cc.). The filtrate and hot water washings from the barium sulfate were concentrated to 50 cc. and placed in the refrigerator. The oxalic acid crystals which were deposited overnight were removed by filtration (3.57 g.). The filtrate, after reduction of its volume to 10 cc., was again placed in the refrigerator. The resulting mixture of large oxalic acid crystals and very small short rods was separated by aspirating the latter together with the mother liquor with a rubber-capped pipet. The fine crystals were collected by centrifugation (180 mg.). The mother liquor was brought to dryness (418 mg.), and the residue triturated with absolute ethanol. The insoluble portion, consisting of inorganic material, was removed by filtration, and the filtrate brought to dryness. The semi-crystalline residue (375 mg.), as well as the original crop of fine rods, was treated separately with boiling concentrated nitric acid (3 cc.) under reflux till the evolution of brown nitrous gases ceased (*ca.* 1 hour). The solutions were concentrated on a hot-plate till crystallization commenced. Since the two batches of crystalline material collected after chilling did not differ materially in melting point and appearance, they were combined (185 mg.) and recrystallized again from boiling concentrated nitric acid, from which it formed small, clear-cut prisms melting with effervescence at 241–245° after softening beginning at 224°. The product did not depress the melting point of the reference specimen (244–248°, soft 224°) obtained from Professor Purves.[4] For analysis the compound was recrystallized from acetone–hexane (m.p. 235–238.5°, soft, 231°).

Anal. Calcd. for $C_{10}H_8O_8$ (254.1): C, 47.25; H, 2.38; neut. equiv., 63.5. Found: C, 47.43; H, 2.49; neut. equiv., 63.1.

The tetramethyl ester IIa was prepared in the usual manner by adding excess ethereal diazomethane to a solution of the acid in dry methanol. After recrystallization from acetone–hexane it melted at 127–130°, alone or in mixture with an authentic preparation of the same melting point.[4]

Anal. Calcd. for $C_{14}H_{14}O_8$ (310.3): C, 54.20; H, 4.55; OCH_3, 40.0. Found: C, 54.40; H, 4.77; OCH_3, 39.6.

The infrared spectrum (Nujol mull) of the ester was identical with that of the reference preparation[4]; 3.45 d, 3.87 ss, 5.76 d, 6.05 s, 6.27 ss, 6.36 ss, 10.48 ss, 10.56 m, 11.13 s, 11.54 m, 11.65 s, 12.79 s, 13.22 md, 13.62 sm, 14.30 s (d = deep, m = medium, s = small, ss = very small).

The 1,4-dimethyl ester IIb was prepared by refluxing a solution of the acid (48 mg.) in 3% methanolic hydrogen chloride (3 cc.) for 6 hours.[3] The esterified material was separated into acidic and neutral fractions in the usual manner. The acidic fraction (47.3 mg.) was recrystallized from acetone–hexane, from which it formed rosettes of platelets melting at 160–167.5°. Further recrystallization failed to raise or sharpen the melting point. In mixture with the reference specimen[4] (m.p. 171–175°) the preparation melted at 167–173°.

Anal. Calcd. for $C_{10}H_{10}O_8$ (282.2): OCH_3, 22.0; neut. equiv., 141. Found: OCH_3, 21.9; neut. equiv., 138.

Ultraviolet Absorption Spectra of Acid II and its Esters.—The ultraviolet absorption spectra of the acid II and its esters IIa and IIb in ethanol are practically identical. They are characterized by a single maximum at 288 mμ (II, IIa) or 290 mμ (IIb), with ε 1260–1300, a shallow minimum at 279 mμ (II) or 275 mμ (IIa, IIb), with ε 1130–1250, and end absorption with an inflection at about 240 mμ, ε 8000–8300, which probably corresponds to the high band at 226 mμ in the spectrum of phthalic acid (butyl phthalate, λ_{max}^{alc} 226 mμ (9500), 272 mμ (1500)). The spectrum of the isomeric ester, tetramethyl benzene-1,2,4,5-tetracarboxylate[8] in ethanol shows the same general pattern (maximum at 291 mμ (2370), minimum at 275 mμ (1780), end absorption with inflection at 240 mμ (9050)). It would thus appear that these features are associated with the number of carboxyl functions (and hence of possible resonance structures) rather than with their distribution over the benzene ring. On the other hand, the segregation in the isomeric ester of the carbomethoxy groups into two pairs insulated from each other by unsubstituted ring carbon atoms seems to impart on each of these groupings something like the character of a separate chromophore, if the nearly twice as high extinction of the maximum over that of the corresponding band of the 1,2,3,4-substituted ester IIa can be so interpreted.

The authors are indebted to Mr. Joseph A. Alicino and his associates for the microanalyses, and to Dr. Nettie Coy for the ultraviolet and infrared measurements.

(8) We are greatly indebted to Prof. R. T. Arnold of the University of Minnesota for making available to us a sample of this ester.

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COMMUNICATIONS TO THE EDITOR

N-PANTOTHENYLCYSTEINE AS A PRECURSOR FOR PANTETHEINE AND COENZYME A

Sir:

Pantetheine is readily converted *in vivo* to coenzyme A (CoA), and the intermediate reactions involved are now known.[1] In contrast, nothing is known of the mechanisms by which pantetheine arises from pantothenic acid. A study of this transformation revealed that in the presence of adenosine triphosphate and cysteine, extracts of acetone-dried cells of *Proteus morganii* transform pantothenate to a compound (I) essentially inactive in replacing pantothenate for *Saccharomyces carlsbergensis*, *Lactobacillus arabinosus* and *Lactobacillus casei*.[2] Pantethine was not formed.[2] However, the product (I) formed was considerably more active in promoting growth of *Acetobacter suboxydans*[2] than an amount of pantothenate equal to that inactivated by the enzyme preparation.

Since cysteine could not be replaced by β-mercaptoethylamine in the inactivation reaction with *P. morganii*, it appeared that I might be N-pantothenylcysteine (II). The corresponding disulfide,

(1) G. D. Novelli and M. H. Hoagland, Abstract 26C, 123rd Meeting, Am. Chem. Soc., Los Angeles, March, 1953; G. D. Novelli, *Fed. Proc.*, in press.

(2) Free pantothenate was determined by assay with *S. carlsbergensis*; pantethine was determined with *Lactobacillus helveticus* 80 (J. Craig and E. E. Snell, *J. Bact.*, **61**, 283 (1951)) before and after digestion with intestinal phosphatase. Assays for pantothenate activity with *A. suboxidans* were conducted in the medium of L. A. Underkofler, A. C. Banz and W. H. Peterson (*J. Bact.*, **45**, 183 (1943)).

TABLE I

THE COMPARATIVE ACTIVITIES OF PANTOTHENIC ACID AND BIS-[N-(PANTOTHENYL)]-L-CYSTINE FOR *Acetobacter suboxydans*

Compound tested	Amount required for half maximum growth, μM. × 10³ per 10 ml.
Calcium pantothenate	11.2
Calcium pantothenate + sulfhydryl compound[a]	11.2
Bis-[N-(pantothenyl)]-L-cystine (III)	2.0
Bis-[N-(pantothenyl)]- + sulfhydryl compound	0.7

[a] Cysteine, β-mercaptoethylamine, thiomalic acid and sodium thioglycolate were equally effective. Each compound was added to III at a 200 to 1 molar ratio.

bis-[N-(pantothenyl)]-cystine (III),[3] corresponded closely in biological properties to I. Thus, III was less than 2% as active as pantothenate for *L. arabinosus*, *L. casei* and *S. carlsbergensis*, and less than 2% as active as pantethine for *L. helveticus*. For *A. suboxydans*, however, III was 2 to 3 times as active as pantothenate, and following treatment with any of several sulfhydryl compounds, was about 8 times as active (Table I). The latter did not alter the activity of pantothenate, and hence presumably acted by reducing the disulfide III to the more active thiol, II. Finally, dried cells of *A. suboxydans* convert II to pantetheine (Table II).

TABLE II

THE ENZYMATIC CONVERSION OF N-PANTOTHENYLCYSTEINE TO PANTETHEINE

The complete system contained per 4 ml.: 0.021 μmole of bis-[N-(pantothenyl)]-L-cystine, 35 μmoles of –SH compound (cysteine or β-mercaptoethylamine), 80 mg. of vacuum-dried cells of *A. suboxydans*, and 1 ml. of 0.25 *M* phosphate buffer, *p*H 6.5; incubation was for 3 hours at 30°.

System	Pantetheine formed, μM. × 10³
Complete	2.9
Complete, heated[a]	0.0
Minus bis-[N-(pantothenyl)]-L-cystine	.0
Minus –SH compound	.0
Minus dried cells	.0

[a] Heated at 100° for 5 min. immediately after the addition of the dried cells.

These experiments indicate that pantothenylcysteine is an intermediate in the formation of pantetheine (and hence CoA) from pantothenic acid. The fact that both II and CoA have high activities for *A. suboxydans*, whereas pantethine is no more active than pantothenic acid, may indicate that pantothenylcysteine is phosphorylated before decarboxylation, thus yielding phosphopantetheine (which has high activity for *A. suboxydans*[4]) directly.

Under conditions that liberate the theoretical amount of pantothenate from pantethine, an extract of pigeon liver liberated only 7% of the bound pantothenate from III. If animals can utilize III, its natural occurrence could explain the observations of Lih, *et al.*,[5] that the pantothenate content of some natural materials as indicated by assay with rats was higher than when the material was subjected to enzyme treatment and pantothenate was measured microbiologically.

(3) We are indebted to Drs. J. F. Cavella and R. E. Bowman, Parke, Davis and Co., Ltd., Hounslow, England, for a sample of this compound.

(4) G. D. Novelli, private communication.

(5) H. Lih, T. E. King, H. Higgins, C. A. Baumann and F. M. Strong, *J. Nutrition*, **44**, 361 (1951).

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RECEIVED APRIL 30, 1953

HELICAL POLYPEPTIDE CHAIN CONFIGURATION IN COLLAGEN[1]

Sir:

Methods for testing X-ray diffraction data for evidence of helical molecular chain configuration have recently been provided.[2] Applications have shown that helical structures hitherto proposed do not account for wide-angle diffraction by collagen.[3] We wish to describe specifications for a helix which promises to be satisfactory.

The table lists the commonly observed near-meridional diffractions of kangaroo tail tendon, as well as new ones photographed in a cylindrical camera with specimens tilted at appropriate angles to the incident Cu Kα radiation. Assignment of layer indices (k) further supports the 20 Å. pseudo-period previously suggested.[4]

Observed spacings, d, Å.	k	$b_0 = kd$, Å.	n
∞	0	...	0
9.55	2	19.1	1
5.0	4	20.0	2
3.97	5	19.9	1
2.86	7	20.0	0
2.27	9	20.4	1
1.83	11	20.1	2
1.64	12	19.7	1
1.45	14	20.3	0

A discontinuous helix with 2 turns and 7 roughly equivalent scattering groups per 20 Å. of axial projection should contribute to near-meridional diffraction according to Bessel functions whose orders, n, are shown. The observed layer lines are those for which n is 2 or less (indeed often one or zero). For missing layer lines n is 2 or greater. The higher layers observed with shorter wavelength radiation[5] would have zero n's, and k indices which are multiples of 7.

The approximately seven-fold screw axis does not permit exact hexagonal packing of these chains in fibrils, but there is no evidence requiring this. Pseudo-hexagonal packing, with about 12 Å. between chain centers (dry fibers), accounts for the

(1) Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council. One of us (C. C.) gratefully acknowledges fellowship support by the National Science Foundation.

(2) W. Cochran, F. H. C. Crick and V. Vand, *Acta Cryst.*, **5**, 581 (1952).

(3) L. Pauling and R. B. Corey, *Proc. Roy. Soc.* (*London*), **B141**, 31 (1953).

(4) R. S. Bear, *Advances in Protein Chem.*, **7**, 115, 130 (1952).

(5) M. F. Perutz, *Ann. Repts., Chem. Soc., London*, **48**, 379 (1952).