[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Erythromycin. VIII. Structure of Dihydroerythronolide[1]

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Dihydroerythronolide (I) has been shown to be 3,5,6,9,11,12-hexahydroxy-2,4,6,8,10,12-hexamethylpentadecan-13-olide, a novel compound exhibiting the biogenetic pattern of a "propionate" polymer. Certain features of the stereochemistry of I are discussed.

In earlier papers[2] of this series the isolation of dihydroerythronolide,[2b] a degradation product of erythromycin, was described. This product represents the aglycone portion of dihydroerythromycin and was obtained from erythromycin by reduction of the ketone function with sodium borohydride[3] followed by removal of cladinose[2b,c] and desosamine.[2a] Evidence was presented that dihydroerythronolide, $C_{21}H_{40}O_8$, is a hexahydroxylactone with a conspicuously high C-methyl content (6 to 7); the presence of two α-glycol systems in the molecule was demonstrated by titration with sodium metaperiodate.

In the present communication degradative studies are reported which show that dihydroerythronolide has the structure I.

Oxidation with sodium metaperiodate was applied to I as well as to the corresponding pentadecanoic acid (II) which was obtained from I *in situ* by mild alkaline hydrolysis (Fig. 1.) Cleavage of I[4] with two moles of oxidant afforded a sirupy mixture of the ketoaldehyde (III, C_9-fragment) and the aldehydoester (IV, C_7OOC_5-fragment). Oxidation of the acid (II) with three moles of periodate led to isolation of the individual C_9-fragment and of two smaller products, propionaldehyde and acetic acid. Because of their particular nature these larger fragments (III and IV) could not be purified by the usual techniques such as crystallization and distillation. The mixture of III and IV, as well as the C_9-fragment III individually, has therefore been converted to a series of well characterized, identified transformation products by means of reductive, oxidative and alkaline reagents (Figs. 2 and 3). It should be stressed that consideration of these transformation products *collectively* rather than individually furnishes proof for structures III and IV.

Catalytic reduction of the mixture of III and IV (Fig. 2) in the presence of small amounts of ferrous chloride followed by mild alkaline hydrolysis yielded three products, the liquid C_9-carbinol V (Fig. 3), C_7-hydroxylactone A VI, and low-melting, crystalline C_5-diol VII. The procedure used to isolate V, VI and VII as described in the Experimental section coupled with the fact that they were obtained in reasonable yields warrants the conclusion that these three products individually derive from the corresponding C_9-, C_7- and C_5-segments of I. These products (V, VI and VII) thus constitute an adequate material balance for this two-stage reaction and they account for all twenty-one carbon atoms of I.

The C_9-carbinol V derived from the C_9-fragment will be discussed in a later section. C_7-Hydroxylactone A VI is undoubtedly formed from the aldehydo-acid portion of IV by reduction of its aldehyde function and lactonization under the influence of catalytic amounts of hydrochloric acid derived from the ferrous chloride present. Reduction of this optically active lactone VI with lithium aluminum hydride gave a crystalline *meso*-2,4-dimethylpentane-1,3,5-triol (VIII) identical with a synthetic sample (Fig. 4) obtained by reduction of α,α'-dimethyl-β-hydroxyglutaric acid (IX)[5] or the corresponding O-acetyl anhydride.[5] This isolation of a *meso* form of the triol VIII establishes a *cis* relationship of the two C-methyl substituents in the precursor lactone VI. Since C_7-hydroxylactone A VI contains the carboxyl group of I, it must represent the first seven carbon atoms of I. As a consequence, the *cis* relationship of the methyl substituents in lactone A VI demonstrates the identity of configurations of C-2 and C-4[6] bearing these two methyl groups in the parent molecule I. Conversion of the lactone VI to a hydrazide X and regeneration of the lactone with dilute hydrochloric acid served as a purification of VI and added valuable optical activity data for stereochemical study.

Turning to the third reduction product VII, this optically active C_5-diol reacted with sodium metaperiodate to give propionaldehyde which was iso-

(1) Part of the work presented in this paper has been reported in a preliminary communication, Paul F. Wiley, Koert Gerzon, Edwin H. Flynn, Max V. Sigal, Jr., U. Carol Quarck and Ollidene Weaver, THIS JOURNAL, **77**, 3677 (1955).

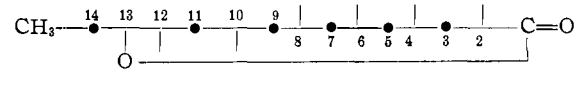
(2) (a) Edwin H. Flynn, Max V. Sigal, Jr., Paul F. Wiley and Koert Gerzon, *ibid.*, **76**, 3121 (1954); (b) Max V. Sigal, Jr., Paul F. Wiley, Koert Gerzon, Edwin H. Flynn, U. Carol Quarck and Ollidene Weaver, *ibid.*, **78**, 388 (1956); (c) Paul F. Wiley and Ollidene Weaver, *ibid.*, **78**, 808 (1956).

(3) Mild acid hydrolysis of dihydroerythromycin led to the isolation of x-O-desosaminyl-dihydroerythronolide (see ref. 2b, formula V) as the sole product. It is therefore concluded that the reduction step which produces the new hydroxyl function proceeds in a stereospecific manner. From information contained in this and previous papers it can be inferred that this "unnatural" hydroxyl group is located at C-9 (see footnote 6) in I; direct evidence in support of this inference will be presented in a future publication dealing with the investigation of erythromycylamine, the product of reductive amination of erythromycin.

(4) The dihydroerythronolide employed in these studies usually contained from 5 to 10% of dehydration product A.[2b] This product, $C_{21}H_{38}O_7$, formed from I by the loss of one mole of water, was shown to be unaffected by periodate reagent. Any lower molecular weight species obtained with this reagent must, therefore, have originated from I. Wherever necessary, results have been confirmed by using I purified by chromatography.

(5) S. Reformatzki, *Ber.*, **28**, 3263 (1895).

(6) The numbering used in this discussion is indicated in the schematic formula



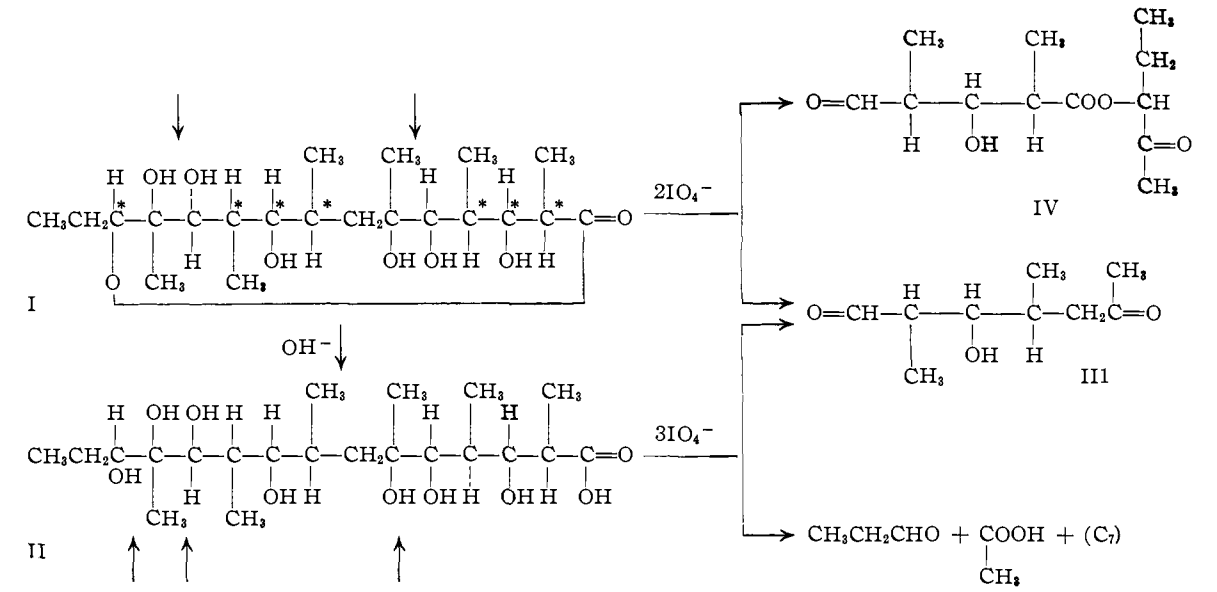
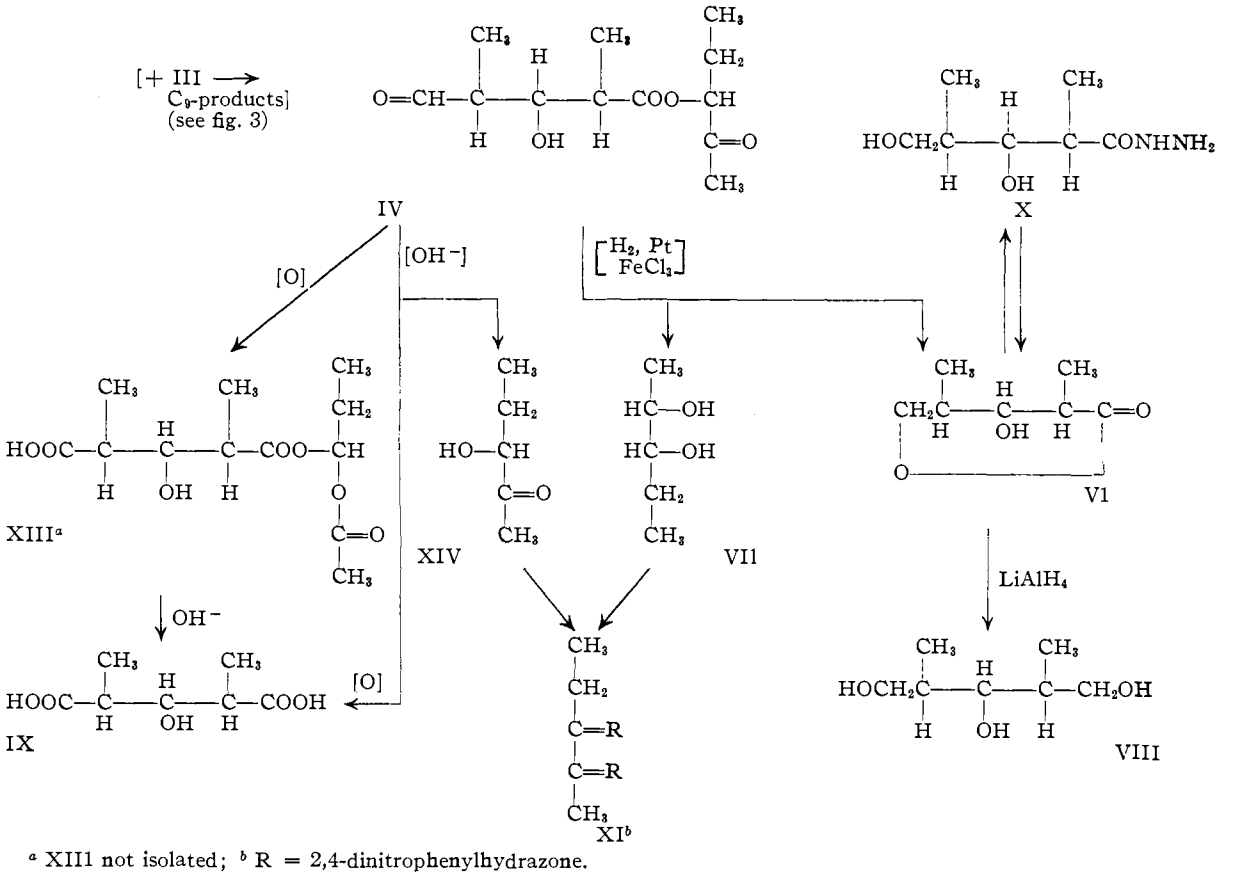


Fig. 1.



[a] XIII not isolated; [b] R = 2,4-dinitrophenylhydrazone.

Fig. 2.

lated as its 2,4-dinitrophenylhydrazone. Oxidation of VII with bromine gave pentane-2,3-dione which was isolated as the 2,4-dinitrophenylosazone XI identical with an authentic sample. These observations define VII as a pentane-2,3-diol. Lucas[7] has reported the synthesis and refractive indices for the non-crystalline *dl-erythro*-pentane-2,3-diol (n^{20}D 1.4431) as well as for the *dl-threo* form (n^{20}D 1.4320). The observed refractive index of VII in the liquid state (n^{25}D 1.4402) together with its isolation as a crystalline product strongly suggests that VII represents one of the enantiomorphs of the *erythro* form of this diol.

Oxidation of the mixture of III and IV with

(7) H. J. Lucas, M. J. Schlatter and R. C. Jones, THIS JOURNAL, **63**, 22 (1941).

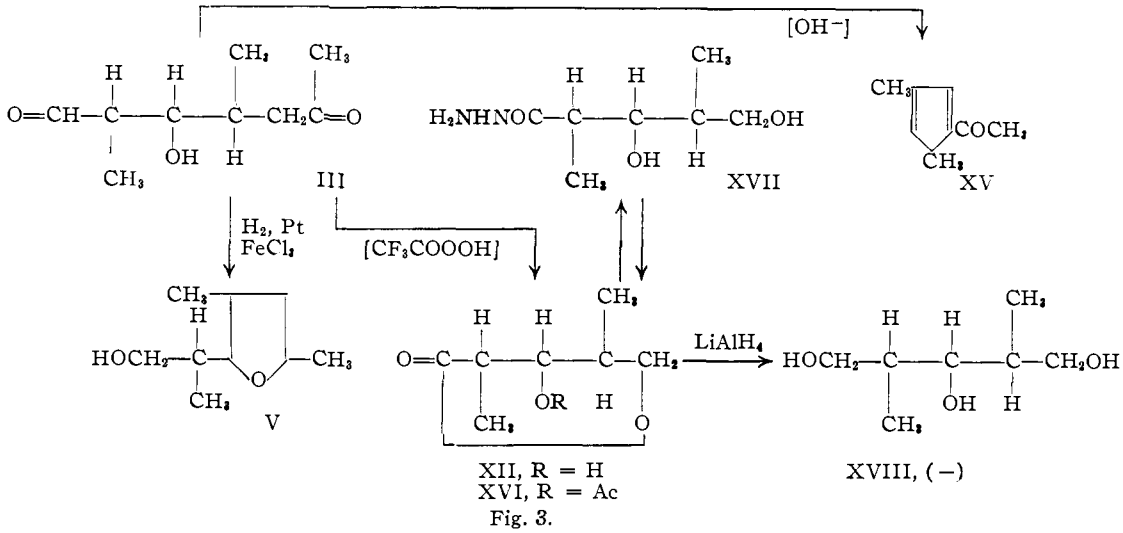


Fig. 3.

peroxytrifluoroacetic acid (Fig. 2) according to the technique of Emmons and Lucas[8] followed by mild alkaline treatment afforded two crystalline products, a new C_7-hydroxylactone B XII (Fig. 3), differing in physical properties from the stereoisomeric lactone A VI, and a *meso*-α,α'-dimethyl-β-hydroxyglutaric acid (IX) identical with the synthetic sample mentioned above. In view of the oxidative nature of this two-stage reaction these products (XII and IX) must originate from the C_9- and C_7-fragments, respectively; in this reaction the products derived from the C_5-fragment (*i.e.*, propionaldehyde and acetic acid) were not sought, but the odor of propionaldehyde was strongly in evidence when the mixture was treated with alkali. Hydrolysis of the presumed intermediate acylal XIII would account for this observation. Discussion of C_7-hydroxylactone B XII will be deferred until a later section dealing with the individual C_9-fragment.

Mild alkaline treatment of the mixture of III and IV (Fig. 2) afforded the steam-volatile, carbonyl-containing C_5-compound XIV which was isolated as the corresponding 2,4-dinitrophenylosazone XI, identical with the sample obtained earlier from the C_5-diol VII. The distillate containing XIV did not form a precipitate in the presence of a nickel salt and hydroxylamine but did form such a precipitate after oxidation with ferric chloride. These observations define XIV as an α-hydroxyketone which must be pentan-3-ol-2-one (XIV) or the isomeric pentan-2-ol-3-one. Survival of such an α-hydroxyketone (XIV or its isomer) during periodate cleavage of I coupled with its liberation by dilute alkali establishes in IV the presence of the ester function linking the C_7-aldehydoacid through its carboxyl function to the hydroxyl group of this hydroxyketone. Also, because of concurrent formation of the C_9-ketoaldehyde III in this cleavage reaction, the aldehyde and ketone function of the C_7OOC_5-ester IV cannot both originate from the same α-glycol system in I. In other words, the C_5-hydroxyketone (XIV or its isomer) isolated here constitutes conclusive evidence that the lactone function in dihydroerythronolide involves the oxygen atom on C-13 adjacent to the C-12,11-α-glycol system. *The isolated C_5-hydroxyketone thus becomes the key degradation product which furnishes unequivocal proof of the multi(14)-membered nature of the lactone ring.*

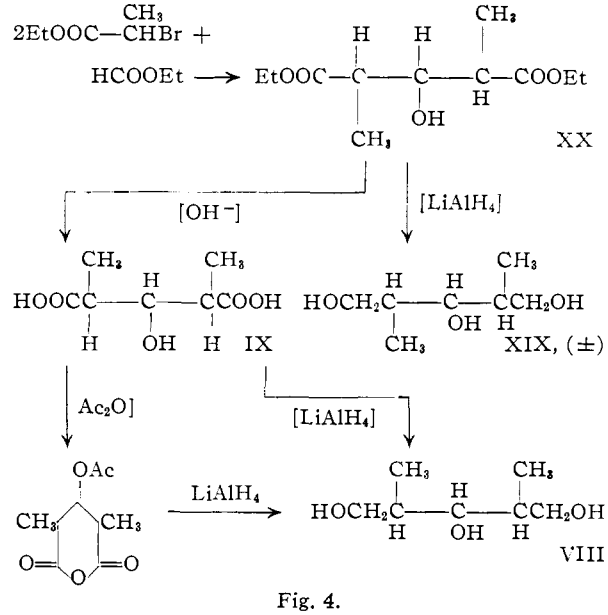
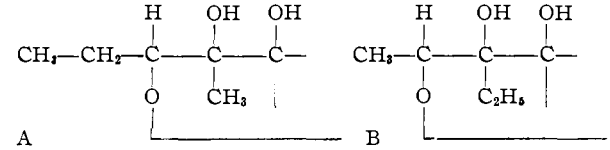


Fig. 4.

No effort was made to determine whether the isolated C_5-hydroxyketone actually is XIV or its isomer since a base-catalyzed ketone–alcohol interchange might have occurred in the course of its isolation. As a consequence two possible structures (A and B) can be written for the terminal six-

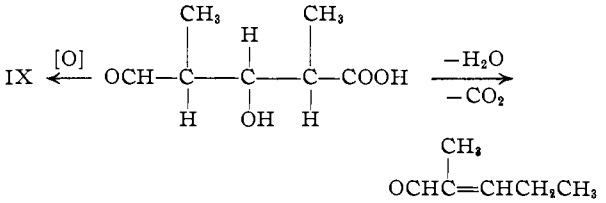


carbon segment of I. A decision in favor of the first structure (A) is readily made on the basis of the isolation and identification of propionaldehyde

(8) W. D. Emmons and G. B. Lucas, THIS JOURNAL, 77, 2287 (1955).

(as its 2,4-dinitrophenylhydrazone) and of acetic acid (as its *p*-bromophenacyl ester) from oxidation of the pentadecanoic acid II with three moles of sodium metaperiodate.[9]

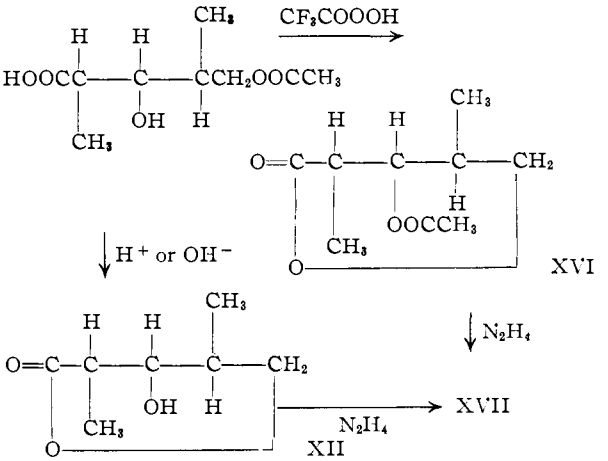
When the C_5-hydroxyketone formed by mild alkaline treatment of III and IV was removed by extraction, a second steam-volatile carbonyl compound was obtained which could be identified as 2-methyl-2-pentenal by means of its 2,4-dinitrophenylhydrazone. A two-step reaction involving dehydration of the intermediate C_7-aldehydoacid followed by decarboxylation provides a rational



explanation for the formation of this unsaturated aldehyde. A small amount of *meso*-α,α'-dimethyl-β-hydroxyglutaric acid (IX) also was obtainable from the alkaline distilland in the form of its *bis*-(*p*-bromophenacyl) ester; the formation of IX presumably entails air oxidation of the same C_7-aldehydoacid.

The C_9-fragment III as obtained from periodate cleavage of II is essentially free of the other degradation products formed,[10] and this preparation, therefore, was used to gain further evidence concerning its structure (Fig. 3). The sirupy material III gave a positive iodoform reaction indicating an acetyl or a potential acetyl group. It formed a bis-(2,4-dinitrophenylhydrazone) of the approximate composition $C_{21}H_{22-24}N_8O_{8-9}$ demonstrating the presence in III of two carbonyl functions; formation of this hydrazone probably involves elimination of the β-hydroxyl function. Treatment of III with dilute sodium hydroxide gave a steam-volatile carbonyl compound XV which formed a black 2,4-dinitrophenylhydrazone of the composition $C_{15}H_{16}N_4O_4$, thus confirming the formulation of III as a fragment containing nine carbon atoms. The ultraviolet spectrum of this latter derivative had a maximum at 402 mμ, ϵ 75,800 consistent[11] with an α,β-γ,δ-unsaturated 2,4-dinitrophenylhydrazone. If XV has the postulated structure of an acetyldimethylcyclopentadiene it should be devoid of the asymmetry of III and, therefore, more amenable to synthesis than III itself. Thus far the attempts to synthesize XV have been unsuccessful.

Oxidation of III with peroxytrifluoroacetic acid[8] yielded two neutral crystalline products, C_7-hydroxylactone B XII isolated previously and its O-acetyl derivative XVI. Isolation of this acetyl compound further confirms the 9-carbon nature of III and establishes the presence in III of a methyl ketone[12] and of an aldehyde function.[13]



Hydrazinolysis of O-acetyl-lactone B XVI gave the C_7-hydrazide B XVII which upon treatment with dilute hydrochloric acid regenerated lactone B XII. The chemical and physical properties of XII and XVII as reported in the Experimental section are in agreement with the structures as written.

The physical properties of lactone B XII and its hydrazide XVII, in accordance with their formulation as diastereoisomers, differ from those of lactone A VI and its hydrazide X. In this respect it is of interest to note that stereochemical differences between the lactones XII and VI apparently result in a small but definite difference in pK'_a values of the respective carboxyl functions in aqueous solution (4.53 for XII, 4.25 for VI). It is further noted that isolation of diastereoisomeric degradation products, here the two lactones and their respective transformation products, from different parts of the same molecule is of rare if not novel occurrence outside the fields of carbohydrate or protein chemistry.

Reduction of lactone B XII with lithium aluminum hydride furnished the crystalline, *optically active* C_7-triol XVIII. The chemical and physical properties of XVIII in conjunction with those of the precursor lactone XII indicate that this triol is one of the enantiomorphic forms of 2,4-dimethylpentane-1,3,5-triol. The observation was made that the infrared spectrum of the optically active C_7-triol in acetonitrile (and in chloroform) solution was very similar but not identical with that of the diastereoisomeric *meso*-triol VIII. Direct evidence for the structure of the active triol XVIII was found in the observed identity of its infrared

(9) This experiment was carried out with dihydroerythronolide purified by chromatography. Titration of the acid II with sodium metaperiodate revealed almost instantaneous uptake of three moles of reagent followed by a slow uptake of one additional mole. It is believed that this fourth mole is utilized in a secondary oxidation reaction involving the C_7-aldehydoacid.

(10) The C_9-fragment generated in this reaction could not be obtained in a satisfactory state of purity; no characterizable derivatives could be prepared.

(11) E. A. Braude and E. R. H. Jones, *J. Chem. Soc.*, 498 (1945).

(12) Formation of the acetyl derivative XVI need not be seen as the result of an intramolecular acyl migration. Lactonization of the primary acidic reaction product releases acetic acid which then can act as an acylating agent under influence of trifluoroacetic acid or of small amounts of trifluoroacetic anhydride. The existence of this postulated primary reaction product is supported by the isolation of a second, acidic fraction from peroxidation of III which could be converted by either aqueous acid or base to the crystalline lactone B XII or the corresponding hydrazide XVII.

(13) Peroxytrifluoroacetic acid has been found to convert enanthaldehyde to the corresponding carboxylic acid in good yield (see Experimental section).

spectrum with that of a synthetic racemic[14] sample of this triol XIX obtained by reduction of diethyl α,α'-dimethyl-β-hydroxyglutarate[5,15] (XX) with lithium aluminum hydride (Fig. 4). The refractive indices of the active triol (XVIII, n^{25}D 1.4756) and of the racemic triol (XIX, n^{25}D 1.4752) in the molten state are likewise in very close agreement, while differing appreciably from the value of this constant for the *meso*-triol (VIII, n^{25}D 1.4827). Inasmuch as the X-ray powder pattern and the melting point of the racemic triol XIX differ from those of the active triol XVIII, XIX must be present as a racemic compound.

Identification of the triol XVIII establishes the structure of its precursor lactone XII and of its acetyl derivative XVI. The structure of the latter product in turn shows the C_9-fragment to be correctly represented by III.[16] Considering this structure (III) the reduction product $C_9H_{18}O_2$ mentioned previously is best formulated as the substituted tetrahydrofurfurylcarbinol V. Analytical and physical data support this formulation as does the fact that V does not give the iodoform test given by the precursor III. The formation of V probably involves hydrogenolysis of the intermediate hemi-ketal,

$$HOCH_2CH(CH_3)\underset{\llcorner\!\!-\!\!O\!\!-\!\!\lrcorner}{CHCH(CH_3)CH_2C(OH)CH_3}$$

In dihydroerythronolide (I) the attachment of the C_9-segment to the C_7OOC_5-segment must of necessity involve the two pairs of carbonyl carbons arising from periodate oxidation. This attachment is effected in the manner shown in I. It is recognized, however, that inverse insertion of this C_9-segment leading to an isomeric structure (Ia,

Ia

schematically) has not been ruled out by the degradative studies reported here. Evidence obtained in the course of such studies with erythromycin and erythralosamine[17] substantiate structure I. This structure strongly suggests a biogenetic origin from three-carbon (propionate?) precursor units in continual "head-to-center" linkage. The alternate structure Ia would be irregular in the sense that it alternates the "head-to-center" arrangement with a "head-to-head" and a "center-to-center" linkage.

In summary, the identity as well as the mode of formation of the divers degradation products described here, when considered collectively, inevitably leads to formulation of structure I for dihydroerythronolide. The identity of lactone ring size in I with that in erythromycin, and the definite placement in the latter of desosamine (at C-5 or C-6), cladinose (at C-3, C-5, or C-6) and the ketone function (at C-9) remain. Evidence concerning these points will be forthcoming shortly.

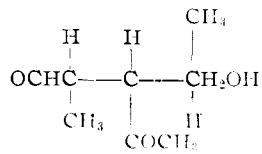
Stereochemistry.—A knowledge of the stereochemistry of the various crystalline degradation products (VI, VII, VIII, X, XII, XVII and XVIII) obtained from I under non-racemizing conditions permits certain tentative conclusions[18] to be drawn concerning the stereochemistry of dihydroerythronolide. Thus a tentative assignment can be made of the configuration of some of the asymmetric carbon atoms, namely, C-2, C-3, C-4, C-8, C-9, C-10 and C-13. In formula I only these carbon atoms (which have been marked with an asterisk) are intended to portray an actual configuration[19] for which there is some experimental support. The configurations of the remaining asymmetric carbons (C-5, C-6, C-11 and C-12) have been represented on a more or less arbitrary basis. The pertinent data concerning optical rotation are summarized in Table I.

The optically inactive C_7-triol VIII (Fig. 2), a new example of a compound containing a pseudo-asymmetric carbon, must represent one of the two possible *meso* forms. No direct evidence is available to decide whether this triol has the *xylo* configuration shown in VIII or the alternate *ribo* configuration (hydroxyl group inverted). However, in the C_7-lactone A VI a *trans* relationship of the β-hydroxyl group to the adjacent methyl groups is suggested by its relative stability with respect to dehydration in the course of acid and base treatment or of distillation of this lactone.

(14) Theoretically, it is not impossible that the synthetic sample XIX represents the second *meso* form of the triol, even though the identity of its spectrum and that of the active triol XVIII militates against this possibility. This uncertainty can only be satisfactorily solved by a resolution of the synthetic material. This uncertainty, however, does in no way detract from the validity of the structural arguments used here: the identity of the spectra of XVIII and XIX establishes the gross structure of XVIII while the optical activity observed for this degradation product defines its enantiomorphic nature.

(15) Since the infrared spectrum of the crystalline, synthetic triol XIX and that of the distilled residue from the mother liquor were found to be identical, it is concluded that the synthetic triol as well as the di-ester XX from which it was obtained consist largely of one (racemic) form with little if any of the respective *meso* species (VIII and diethyl ester of IX) present. It follows that saponification of the racemic diester XX must involve epimerization to yield the *meso* form IX of the glutaric acid.

(16) The alternative structure for the C_9-fragment which upon



treatment with peroxytrifluoroacetic acid could likewise produce the acetyl derivative XVI can be ruled out because of its inability to give rise to an α,β-γ,δ-unsaturated carbonyl system as found in the postulated cyclopentadiene derivative XV.

(17) Details of this work are to be reported in a forthcoming publication. Chromium trioxide oxidation of erythralosamine [see paper I in this series, THIS JOURNAL, **76**, 3121 (1954)] followed by acid treatment has furnished a dilactone $C_{13}H_{18}O_4$ believed to represent the first thirteen carbon atoms of I. The newly formed, second carboxy group in this dilactone is thought to be derived from the ketone function of C-9 in erythronolide. Insertion of the C_9-segment in the lactone ring in the inverse manner gives a structural analog of Ia for erythronolide which cannot account for the formation of a dilactone of this size.

(18) Further work here and by Dr. C. C. Price at the University of Pennsylvania is in progress to obtain additional experimental support for the configurations assigned. Dr. Price's contribution toward the interpretation of the stereochemical data is gratefully acknowledged.

(19) With the exception of the C_5-diol (VII) and the C_5-fragment in IV the formula used in Figs. 1–3 are drawn with the understanding that they represent the correct stereochemical configuration only after a rotation in the plane of the paper of 90° clockwise for lactone B (XII) and its hydrazide (XVII), counterclockwise for I, II, and the remaining degradation products named here. The nomenclature used throughout is that proposed by Klyne, *Chem. and Ind.*, 1022 (1951).

TABLE I[a]

Compound		t, °C.	$[\alpha]_D$
Dihydroerythronolide	I	27	+ 9.5°
C_7-Lactone A	VI	25	− 5.0
C_7-Hydrazide A	X	25	−42.0
C_7-Triol (*meso*)	VIII	25	+ 0.1
C_7-Diacid[b]	IX	33	− 0.1
C_7-Lactone B	XII	25	+56.1
C_7-Hydrazide B	XVII	32	+ 6.7
C_7-Triol (*active*)	XVIII	25	−14.0
C_6-Diol	VII	28	+20.0

[a] The solvent for VII was water; for all other compounds methanol was used. [b] The *meso* character of the glutaric acid IX is demonstrated by its reduction to the *meso*-triol VIII: Formation of IX from I *via* the acylal XIII does not preclude the possibility of epimerization during the saponification step. Isolation of IX from I, therefore, does not constitute reliable stereochemical evidence.

This assignment is supported by the survival of the hydroxyl function at C-3 in I in the course of its formation from x-O-desosaminyldihydroerythronolide under acidic conditions.[2b] On the basis of this conjecture[18] the *xylo* configuration becomes the favored one for lactone A VI as well as for the corresponding hydrazide X and the *meso*-triol VIII.

The optically active C_7-triol must be the isomer XVIII (Fig. 3) or its enantiomorph. Theoretically, the isomer XVIII can be derived either from the *lyxo* form of its precursor lactone B XII or from the *arabo* isomer (hydroxyl group inverted). A tentative assignment in favor of the *lyxo*-configuration XII can be arrived at on the basis of optical rotations of lactones A and B, of the corresponding hydrazides, and of the active C_7-triol, as follows (Fig. 5). A rotational shift of −37° for the lactone–hydrazide conversion in the A-series compares favorably in sign as well as in magnitude with the corresponding shift of −49° in the B-series. Considerable information is available correlating optical rotational data and configuration of asymmetric molecules. When applying some such correlations[20] as the Hydrazide rule[20a] and also Marker's rule[20b] to the present group of compounds it can be concluded that the α-carbon atoms in the two lactones (and in the hydrazides) have identical configuration and most likely belong to the L-series.[19] Furthermore, the difference in rotation between lactones A and B amounts to about 61°, while the difference between the hydrazides A and B is about 49° in the same direction. These differences accompanying the change in configuration between the A- and the B-series must be caused by either an inversion of the γ-methyl substituent alone to give the *arabo* isomer or of both the γ-methyl and the β-hydroxy substituents to give the *lyxo* configuration portrayed in XII and XVII. The individual contribution of the asymmetric center $HOCH_2CH(CH_3)$– can be estimated to be about 7°, since two such centers of the same configuration produce a rotation of 14° in the active C_7-triol XVIII. An inversion of configuration only at the γ-carbon of lactone A or its hydrazide (giving rise to the corresponding *arabo* isomers) would therefore be predicted to produce a change in rotation of about

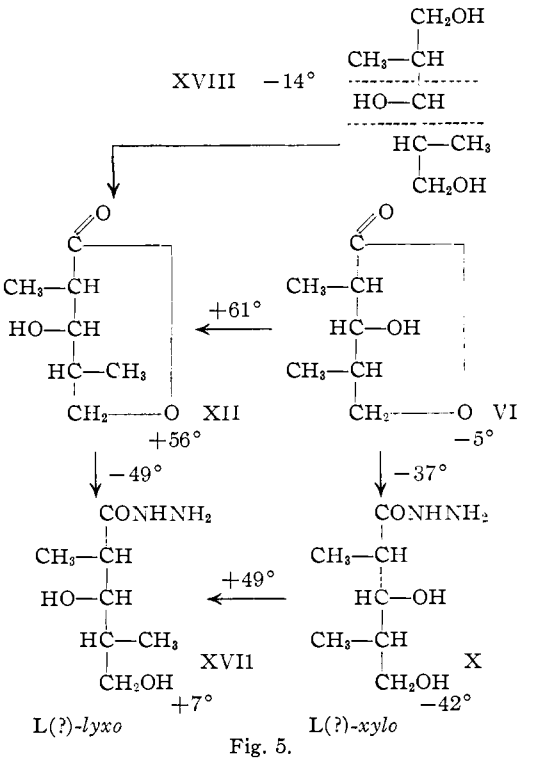


Fig. 5.

twice the value for this asymmetric center, namely, about 14°. To account for the observed changes of 61 and 49°, respectively, it is evident that an inversion must have occurred both at the methyl substituted γ-carbon and at the hydroxyl substituted β-carbon. We therefore propose that the lactone A VI represents the L(?)-*xylo* and, consequently, lactone B XII the L(?)-*lyxo* isomer of 3-5-dihydroxy-2,4-dimethylvaleric acid-5-lactone.[21]

(20) (a) Levene, *J. Biol. Chem.*, **23**, 145 (1915); (b) R. E. Marker, THIS JOURNAL, **58**, 976 (1936); (c) K. Freudenberg, "Stereochemie," Vol. II, Deuticke Verlag, Leipzig, 1933, pp. 675 ff.

(21) The use of the L-designation in conjunction with the prefixes *xylo* and *lyxo*- leads to ambiguity. When using these prefixes in the field of sugar chemistry the assignment of a compound to the L- or D-series is traditionally based on the configuration of the penultimate carbon. On the other hand, when discussing α-substituted fatty acids the assignment of a particular isomer to the L- or D-series is usually based on the configuration of the α-carbon. The latter convention appears to be the more informative and convenient one for use with the present series of compounds.

The nomenclature used for this lactone is consistent with that employed by *Chemical Abstracts*. According to this nomenclature the correct expression for dihydroerythronolide would be 3,5,6,9,11,12,13-heptahydroxy-2,4,6,8,10,12-hexamethylpentadecanoic acid-13-lactone; the expression used for I in the present paper (see Abstract) utilizes the suffix -olide and brings out more clearly the relation to the trivial name dihydroerythronolide. Also this name, hexahydroxyhexamethylpentadecan-13-olide, correctly conveys the fact that I is a *hexa*hydroxy-substituted lactone and therefore appears to be the preferred name for the purpose of this discussion.

NOTE ADDED IN PROOF.—In more recent studies, (+)-2L-methylbutyric acid, obtained by oxidation of (−)-2L-methylbutanol ("active amyl alcohol") was converted with diazomethane to the corresponding methyl ester ($\alpha^{25}D$ + 19.8°; *c*, 1 in methanol) which upon treatment with hydrazine gave the crystalline (+)-2L-methylbutyric acid hydrazide ($\alpha^{25}D$ +38.0°; *c*, 2 in methanol). As the L-configuration of the acid has been established unequivocally (see W. Klyne, "Progress in Stereochemistry," Academic Press, Inc., London, 1954, p. 188), it follows that the change of rotation between ester and hydrazide —"Hydrazide Shift"—is *positive* (+18.2°) for a 2L-methyl substituted acid. On the basis of this observation it would appear that the D-configuration should be assigned to the *xylo*- and *lyxo*- lactones VI and XII (negative hydrazide shift), instead of the tentative L(?)-assignment made previously. A definite assignment will be made at a later date on the basis of experimental work in progress (see footnote 18).

The help of Dr. C. Djerassi who supplied the optically active methylbutyric acid is gratefully acknowledged.

Continuing the above line of reasoning it follows that the active C_7-triol must be the L-enantiomorph XVIII. Judging by some of the correlations of configuration and rotation mentioned earlier[20b,c] this L-designation appears to be compatible with the levorotation observed for this triol.

In transposing the information gathered for lactones A and B (Fig. 6) to the stereochemistry of I, the following conclusions can be reached. Three of the methyl-bearing carbon atoms, C-2, C-4 and C-8 have the same L-configuration, while a fourth, C-10, has the opposite, that is D-configuration. The two hydroxyl-bearing carbon atoms C-3 and C-9 must have the same, namely, the D-configuration. It is recognized, however, that this D-assignment, but not the identity, of configuration depends on the correctness of the assumed *xylo* configuration for lactone A (VI).

The formulation of the C_5-diol VII as an enantiomorph of *erythro*-pentane-2,3-diol implies a stereospecific reduction of the ketone carbonyl in the ester IV producing a new secondary hydroxyl function at C-2 (Fig. 2), the configuration of which bears no known relationship to the original configuration at C-12 in I. On the other hand, an inspection of the mode of formation of this diol VII from I *via* the ester IV shows the configuration of C-3 to be the original configuration at C-13 in I. On the basis of published evidence[20c] correlating the sign of optical rotation and configuration of asymmetric secondary alcohols, the D-configuration can be tentatively assigned to C-3 in this dextrorotatory diol VII and thence to C-13 bearing the lactone ring in I (Fig. 6).

The carbon-atom pairs C-5,6 and C-11,12 represent the two secondary-tertiary α-glycol systems in I, and the stereochemistry of these four remaining asymmetric centers has thus far been inaccessible. Arbitrarily, these two pairs of carbon atoms have been given opposite configurations as drawn in I. In so doing the molecule as a whole increasingly acquires the nature of a *meso* compound in accord with the observed low optical rotatory value ($\alpha^{27}D$ 9.5°) of I.

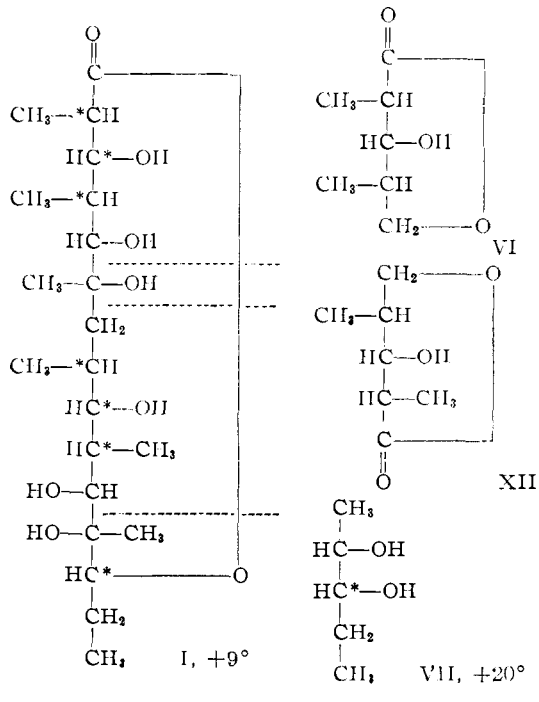


Fig. 6.

In summary, structure I (Fig. 6) represents the stereochemical configuration of those seven carbon atoms (marked with asterisk) for which evidence has been acquired. In terms of the nomenclature recently proposed by Klyne,[19] dihydroerythronolide as portrayed in I can be designated as 3D,5?,6?,9D,11?,12? - hexahydroxy - 2L,4L,6?,8L,10D,12?-hexamethylpentadecan-13D-olide.[22] Future work is planned[18] to obtain further evidence in support of the configuration assigned and to develop routes to those configurations thus far inaccessible.

Biogenetic Aspects.—The conspicuous regularity of seven recurring three-carbon units strongly recalls earlier suggestions concerning a possible participation of propionic acid or its biogenetic equivalents in the formation of certain long chain aliphatic compounds with branched methyl groups.[23] Pertinent examples of such compounds are two acids from tubercle bacilli, tuberculostearic acid,[24] $CH_3(CH_2)_7CH(CH_3)CH_2(CH_2)_7COOH$, and mycoceranic acid,[25] $CH_3(CH_2)_{11}CH(CH_3)CH_2CH(CH_3)CH_2CH(CH_3)COOH$. However, where isolated branched methyl groups are concerned as in tuberculostearic acid, there appears to be no compelling reason to preclude the participation of other proximal precursors, for example of β,β-dimethylacrylic acid.[26] A genuine propionate pattern becomes discernible in mycoceranic acid and now emerges in dihydroerythronolide with complete regularity. This compound is thus the first long-chain aliphatic structure[27] known to obey fully this three-carbon regularity for which we have proposed the name *Propionate rule.*[28] Reported evidence concerning certain related antibiotics of the Erythromycin family, narbomycin,[29] methymycin,[30] picromycin[31]

(22) For the purpose of clarity it appears advisable when applying Klyne's nomenclature to tetrasubstituted carbon atoms (here C-6 and C-12) to name each individual substituent with its proper designation even though this represents an obvious repetition. Also for the purpose of expressing the various stereochemical interrelationships more clearly I has been represented here using the straight-chain formulation instead of the seven-pointed star formula used previously (see E. A. Braude, *Nature*, **176**, 761 (1955), and footnote 28).

(23) (a) R. Robinson, "The Structural Relations of Natural Products," Oxford, Clarendon Press, 1955, p. 7; (b) R. B. Woodward, *Angew. Chem.*, **68**, 19 (1956).

(24) M. A. Spielman, *J. Biol. Chem.*, **106**, 87 (1934); F. S. Prout, J. Cason and A. W. Ingersoll, THIS JOURNAL, **70**, 298 (1948).

(25) G. S. Marks and N. Polgar, *J. Chem. Soc.*, 3851 (1953).

(26) J. Bonner and B. Arreguin, *Arch. Biochem.*, **21**, 109 (1949); **31**, 234 (1951). This acid would appear to be a likely precursor for naturally occurring iso-fatty acids (R. P. Hansen, F. B. Shorland and N. June Cooke, *Biochem. J.*, **58**, 358 (1954)).

(27) A noteworthy example of a six-carbon compound adhering to the propionate pattern is (−)-3-hydroxy-2-methylpentanoic acid isolated from Mycobactin, a growth factor for *M. johnei;* see G. A. Snow, *J. Chem. Soc.*, 4080 (1954).

(28) Paper by K. G. at the XIVth International Congress of Pure and Applied Chemistry at Zurich, July 23, 1955; see also ref. 23a.

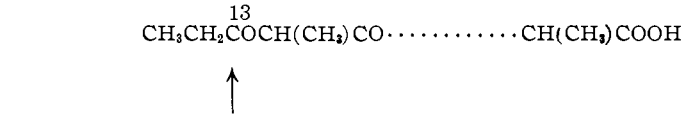
(29) R. Corbaz, L. Ettlinger, E. Gaumann, W. Keller, P. Kradolfer E. Kyburz, L. Neipp, V. Prelog, R. Reusser and H. Zahner, *Helv. Chim. Acta*, **38**, 935 (1955); also personal communication by V. Prelog to K. G.

(30) Carl Djerassi, A. Bowers, R. Hodges and B. Riniker, THIS JOURNAL, **78**, 1733 (1956).

(31) H. Brockmann and R. Oster, *Naturwiss.*, **42**, 155 (1955).

and perhaps others, reveals that the structures of their aglycones adhere partially if not fully to this same rule. It is felt that at least within this group of products of microbiological origin the Propionate rule offers a valuable tool in structure prediction much as does the widely applicable isoprene rule[32] in the terpene field. Fermentation studies in these laboratories using labeled propionic acid are aimed at the experimental verification of its role as a precursor in the biosynthesis of erythronolide.

As has been shown, the propitious arrangement of oxygen functions in I at C-11, C-12 and C-13 has made possible the unequivocal proof of the macrocyclic nature of I. Although isolation of simpler macrocyclic lactones such as pentadecan-15-olide[33] and ambrettolide[33] from plant sources has been reported, the highly substituted dihydroerythronolide appears to represent the first well established example of such a lactone of microbiological origin. Little if any information is available concerning biogenesis of large membered lactones. It has been proposed[34] that macrocyclic ketones be formed by a Dieckmann-type condensation of α,ω-dicarboxylic fatty acids with loss of carbon dioxide. It is conceivable that oxygenation of such odd-membered ketones by peroxides could produce even-membered lactones such as pentadecan-15-olide.[35] An examination of the structure of I tends to show that the biosynthesis of this compound follows a different pathway. The hypothetical intermediate



does not cyclize in the Dieckmann manner by utilizing either of the activated carbon atoms C-12 or C-14. Instead, ring closure to form the 14-membered lactone is apparently effected between the terminal carboxyl group and the oxygen function at C-13 representing the carboxyl group of the first propionate unit.

Acknowledgment.—The authors are grateful to Miss Ollidene Weaver for valuable technical assistance; to Messrs. W. L. Brown, H. L. Hunter, G. M. Maciak and Miss Gloria Beckmann for microanalyses; to Dr. H. Boaz for helpful discussions of the physicochemical data, and to Mrs. Helen Arndt, Miss Martha L. Hofmann, Messrs. J. W. Forbes, D. O. Woolf, Jr., and Paul W. Landis for the absorption spectra and titrations; to Dr. H. Rose and Miss Ann Van Camp for X-ray data. The authors should like to acknowledge the benefit of stimulating discussions with their associates and the senior members of these laboratories.

(32) Wallach, *Ann.*, **238**, 78; **239**, 49 (1887); Ruzicka, "Über Konstitution und Zusammenhänge in der Sesquiterpenreihe," 1928.

(33) M. Kerschbaum, *Ber.*, **60**, 902 (1927).

(34) R. Robinson, *Nature*, **158**, 815 (1946).

(35) An analogous conversion of ring-D ketones in some steroids to the corresponding lactones by certain molds has been reported recently; see J. Fried, R. W. Thoma and A. Klingsberg, THIS JOURNAL, **75**, 5764 (1953); M. F. Murray, B. A. Johnson, R. L. Pederson and A. C. Ott, *ibid.*, **78**, 981 (1956).

Experimental[36]

C_9-Fragment III and C_7OOC_5-Fragment IV from I.—Dihydroerythronolide (15.75 g., 37.5 mM.) was dissolved in 375 ml. of methanol and to this solution was added 375 ml. of water and 750 ml. of a 0.1 *M* sodium metaperiodate solution. The reaction mixture was allowed to stand at room temperature for 22 hours; at this time a sample of the mixture still gave a positive test for periodate ion. The methanol was removed at room temperature under reduced pressure, and the aqueous solution was allowed to stand at 0° for 24 hours. A small amount of sodium iodate which had precipitated was filtered off, and the filtrate was extracted with five 150-ml. portions of chloroform. The combined chloroform extracts were washed with 35 ml. of a saturated sodium chloride solution and dried over magnesium sulfate. The chloroform solution was concentrated under reduced pressure, leaving the mixture of III and IV as a sirupy residue weighing 12.2 g. The infrared spectrum showed hydroxyl absorption at 2.9 μ, aldehyde absorption at 3.6 μ, and intense carbonyl absorption between 5.7 and 5.9 μ. The ultraviolet spectrum contained an absorption maximum at 278 mμ (ϵ about 100 based on I).

C_9-Carbinol V, C_7-Hydroxylactone A VI and C_5-Diol VII by Catalytic Reduction of III + IV.—The above mixture of III and IV (12.2 g.) was dissolved in 30 ml. of methanol, and this solution was added to a suspension of 2 g. of platinum oxide in ethanol which had been reduced with hydrogen in the presence of 6 ml. of a 20% aqueous ferrous chloride solution. The mixture was then subjected to reduction with hydrogen in an Adams apparatus until all carbonyl absorption in the ultraviolet had disappeared (4 days). The ethanolic solution was concentrated to small volume under reduced pressure at room temperature, and the residue was taken up in 400 ml. of chloroform. The chloroform solution was washed with two 5-ml. portions of a saturated sodium bicarbonate solution and with two 5-ml. portions of a saturated sodium chloride solution. The chloroform solution was then filtered with the aid of charcoal and dried over magnesium sulfate. The chloroform was removed at room temperature under reduced pressure, leaving 9 g. of a viscous oily residue, consisting of V, VI, VII and presumably tetrahydro-IV. The residue was diluted with 3 ml. of ether and 3 ml. of petroleum ether,[37] and the solution was allowed to stand for 2 days at 0°. The crystalline product was filtered with suction and freed from adhering oily impurities by drying between filter paper. The yield of material (VI), melting at 85–88°, amounted to 1.0 g. Recrystallization from a mixture of ether and petroleum ether raised the melting point to 87–88°.

The X-ray diffraction pattern as well as the infrared spectrum of this material was identical to those of the analytically pure material described below under "Conversion of C_7-Hydrazide A X to C_7-Lactone A VI."

The filtrate of the crystallization of VI was concentrated to small volume and the oily residue (7.5 g.) was dissolved in 75 ml. of methanol. Saponification was effected by stirring this solution with 15 ml. of 1 *N* sodium hydroxide solution in a closed vessel for 16 hours. The methanol was removed under reduced pressure, and the remaining aqueous alkaline solution was extracted with five 25-ml. portions of chloroform to extract the reduced C_9-fragment V. The combined chloroform extracts were washed with 3 ml. of water, the water wash being added to the alkaline solution. The chloroform solution was then shaken thoroughly with 2.5 ml. of a 20% sodium metaperiodate solution to cleave any diol VII present. A faint aldehyde smell was noticeable; the aqueous layer still gave a positive test for periodate ion. The chloroform solution was washed first with 5 ml. of a saturated sodium chloride solution, then shaken thoroughly with two 2.5-ml. portions of a saturated sodium bisulfite solution, and finally washed with 5 ml. of a saturated sodium carbonate solution and dried over magnesium sulfate. The residue (1.8 g.) remaining after removal of the chloroform was distilled under reduced pressure in the presence of a small amount of anhydrous potassium carbonate.

(36) Melting points were determined on a Kofler micro melting point apparatus. Infrared spectra were obtained with a Beckman IR-2T spectrophotometer or a Baird double-beam recording spectrophotometer. Ultraviolet measurements were made with a Cary recording spectrophotometer.

(37) Throughout the Experimental section the petroleum ether fraction used was Skellysolve B.

The colorless product V ($n^{20}D$ 1.4519, b.p. 87–88° at 5 mm.), did not give an iodoform reaction and did not decolorize bromine in carbon tetrachloride. The infrared absorption spectrum showed a symmetrical hydroxyl absorption band at 2.85 μ; the ultraviolet spectrum was transparent between 220 and 400 mμ.

Anal. Calcd. for $C_9H_{18}O_2$: C, 68.31; H, 11.47; C-CH_3 (3), 28.5; mol. wt., 158. Found: C, 68.15; H, 11.39; C-CH_3, 23.8; mol. wt., 176 (ebull.); $[\alpha]^{26}D$ +15° (*c* 1, in methanol).

By titration of an aliquot with sodium metaperiodate, the above alkaline solution was found to contain 13 millimoles of reactive material (C_5-diol VII). The alkaline solution was extracted continuously with ether for a period of 72 hours, the ether extract was washed once with 5 ml. of a saturated sodium chloride solution and dried over anhydrous potassium carbonate. The residue (0.95 g.) after removal of the ether was distilled under reduced pressure to give a colorless product VII, b.p. 38° at 0.05 mm., $n^{25}D$ 1.4402, which crystallized upon cooling at 0° (X-ray diffraction pattern) but melted again upon being warmed to room temperature (25°). The reported[7] boiling point for the liquid *erythro*-racemate of 2,3-pentanediol is 89° at 10 mm., $n^{20}D$ 1.4431.

Anal. Calcd. for $C_5H_{12}O_2$: C, 57.69; H, 11.54. Found: C, 57.74; H, 11.78; $[\alpha]^{28}D$ +20° (*c* 1, in water).

The remaining aqueous solution containing the sodium salt of lactone A VI was acidified by the addition of cold 20% hydrochloric acid solution to *p*H 1.0 and allowed to stand with 40 ml. of chloroform for 3 hours. The layers were separated and the acidic layer was extracted with six 40-ml. portions of chloroform. The combined chloroform extracts were washed with 5 ml. of a saturated sodium bicarbonate solution and dried over magnesium sulfate. Removal of chloroform under reduced pressure left a crystalline residue. The product was recrystallized from a mixture of chloroform and petroleum ether, yielding 0.75 g. of lactone A VI identical (X-ray diffraction pattern, infrared spectrum) with the earlier sample isolated directly from the reduction mixture of III and IV.

In a second series of experiments designed to obtain maximum yields, the total amount of crystalline A VI obtained from 15.75 g. of I amounted to 1.87 g. (41% of theory); the yield of crude C_9-carbinol V was 1.5 g. (24%) and of distilled product was 1 g. (16%); the total yield of diol VII as determined by titration with sodium metaperiodate was found to be 0.016 mole (41%). In view of the presence of about 10% of dehydration product A in the sample of I used here, these yields must be augmented accordingly.

C_7-Hydrazide A X from C_7-Hydroxylactone A VI.—Lactone A (500 mg.) was dissolved in 1 ml. of absolute methanol. This solution was heated with six drops of anhydrous hydrazine in a closed vessel at 90° for 18 hours. The solvent and excess hydrazine were removed under reduced pressure, and the solid residue was crystallized from a mixture of ethanol and ether to give a nearly quantitative yield of crystalline product, m.p. 139–141°. Recrystallization from the same solvent mixture raised the melting point to 140–141°.

Anal. Calcd. for $C_7H_{16}N_2O_3$: C, 47.73; H, 9.09; N, 15.91. Found: C, 47.96; H, 9.20; N, 16.13; $[\alpha]^{25}D$ −42° (*c* 0.55, in methanol).

Reconversion of C_7-Hydrazide A X to Lactone A VI.—Hydrazide A (230 mg.) was dissolved in 20 ml. of 1*N* hydrochloric acid, and the acidic solution was stirred with 50 ml. of chloroform at room temperature for 18 hours. The layers were separated, and the aqueous layer was extracted with three 25-ml. portions of chloroform. The combined chloroform extracts were washed first with 10 ml. of a saturated sodium chloride solution, with 10 ml. of a saturated sodium bicarbonate solution, and finally with 10 ml. of a saturated sodium chloride solution. The chloroform solution was dried over magnesium sulfate, and the chloroform was removed under reduced pressure to give a residue which readily crystallized upon the addition of a few drops of petroleum ether. Recrystallization from a mixture of chloroform and petroleum ether afforded pure lactone A melting at 88–88.5° with prior sublimation. The product could be distilled without decomposition at 0.05 mm. at a bath temperature of 90°.

The infrared spectrum showed hydroxyl absorption at 2.9 μ and lactone absorption at 5.82 μ. The ultraviolet spectrum was transparent between 220 and 400 mμ. Saponification followed by titration in water revealed a pK'_a of 4.25 with an apparent molecular weight of 144 (calcd. 144).

Anal. Calcd. for $C_7H_{12}O_3$: C, 58.33; H, 8.33; C-CH_3 (2), 20.9. Found: C, 58.27; H, 8.27; C-CH_3, 19.9; $[\alpha]^{27}D$ −5° (*c* 2, in methanol).

***meso*-2,4-Dimethylpentane-1,3,5-triol (VIII) by Reduction of Lactone A VI.**—Lactone A (400 mg.) was dissolved in 50 ml. of anhydrous ether and this solution was added dropwise to a stirred suspension of 1.14 g. of lithium aluminum hydride in 200 ml. of anhydrous ether. The mixture was heated under reflux for one hour after which time excess lithium aluminum hydride was decomposed by addition of 1.2 ml. of water, 0.9 ml. of a 20% sodium hydroxide solution and finally 4.2 ml. of water. The solids were filtered off and washed with three 25-ml. portions of anhydrous ether. The combined ether layers were washed with 5 ml. of a saturated sodium chloride solution, dried over magnesium sulfate and concentrated under reduced pressure. The viscous residue crystallized on standing at 0° yielding 350 mg. (85%) of triol melting at 78–80°. The optical rotation of this triol was zero within experimental error; $[\alpha]^{33}D$ −0.1° (*c* 2, in methanol).

Methylethylglyoxal 2,4-Dinitrophenylosazone (XI) by Oxidation of C_5-Diol VII.—A solution of 50 mg. of 2,3-pentanediol (VII) in 15 ml. of water was added to 80 ml. of water containing 160 mg. of partially dissolved bromine. The reaction mixture was allowed to stand in sunlight for a total of 56 hours during which time the brown color of the solution gradually disappeared. The pale yellow solution was added to 200 ml. of a solution of 2,4-dinitrophenylhydrazine containing 200 mg. of the reagent and 60 ml. of 6 *N* hydrochloric acid. The precipitate that formed was filtered off, digested with hot ethanol for 15 minutes, filtered and dried. The product, m.p. 180–182° with prior sublimation, was identified as the 2,4-dinitrophenylosazone of methylethylglyoxal by a comparison of its X-ray diffraction pattern with that of an authentic sample of this derivative: a mixed melting point was undepressed.

C_5-α-Hydroxyketone XIV and the Corresponding 2,4-Dinitrophenylosazone XI by Mild Alkaline Hydrolysis of III + IV.—The mixture of III and IV obtained as described above from 660 mg. of dihydroerythronolide (I) was suspended in 150 ml. of 0.013 *N* aqueous sodium hydroxide and steam distilled at constant volume; 200 ml. of distillate was collected.

A 25-ml. aliquot of the above distillate was treated with 2 ml. of a 20% aqueous solution of hydroxylamine hydrochloride, 5 ml. of a 20% aqueous solution of sodium acetate and 2 ml. of a 10% aqueous solution of nickel chloride. On standing there was no evidence of the formation of a nickel glyoxime precipitate.

A second 25-ml. aliquot of the above distillate was treated with 15 ml. of a 10% aqueous ferric chloride solution. The mixture was distilled and 12 ml. of distillate was collected. This distillate was treated with 2 ml. of a 20% aqueous solution of hydroxylamine hydrochloride, 5 ml. of a 20% aqueous solution of sodium acetate and 2 ml. of a 10% aqueous solution of nickel chloride. On standing a brownish-red precipitate was formed, characteristic of a nickel glyoxime complex.[38]

The remaining 150 ml. of the above distillate was treated with 0.45 g. of 2,4-dinitrophenylhydrazine, 90 ml. of concentrated hydrochloric acid and 225 ml. of water. After several hours the initial precipitate was removed by filtration and an additional 0.38 g. of 2,4-dinitrophenylhydrazine and 75 ml. of concentrated hydrochloric acid were added to the filtrate. The solution was allowed to stand at room temperature for 2 weeks. The precipitate which formed weighed 240 mg. and after recrystallization from a mixture of pyridine and methanol melted at 275–277°.[11]

Anal. Calcd. for $C_{17}H_{16}N_8O_8$: C, 44.35; H, 3.50; N, 24.34. Found: C, 44.00; H, 3.41; N, 23.72.

The X-ray diffraction pattern of this product (XI) was identical with that of an authentic sample of the 2,4-dinitrophenylosazone of 2,3-pentanedione.[39]

***meso*-α,α'-Dimethyl-β-hydroxyglutaric Acid (IX) and 2-Methyl-2-pentenal by Mild Alkaline Treatment of III +**

(38) C. B. Van Niel, *Biochem. Z.*, **187**, 472 (1927).

(39) Obtained from Forest Products Chemical Co.

IV.—The mixture of III and IV obtained as described above from 2 g. of I was dissolved in 100 ml. of 50% aqueous methanol and 48.0 ml. of 0.1 *N* aqueous sodium hydroxide was added. The alkaline solution was allowed to stand at room temperature for one hour and then adjusted to *p*H 8.0 with 0.1 *N* aqueous hydrochloric acid (5.9 ml. of acid was required; the base consumed equalled 88.5% of theory for one carboxyl group). The methanol was removed under reduced pressure, and the aqueous solution was extracted with four 50-ml. portions of chloroform to remove neutral products (III and XIV).

The aqueous solution was then steam distilled at constant volume. The first 100 ml. of distillate was treated with 350 ml. of Brady reagent, and a precipitate was obtained which weighed 400 mg. After two crystallizations from ethanol this product melted at 161–163.5°, and its X-ray diffraction pattern was identical with that of an authentic sample of the 2,4-dinitrophenylhydrazone of 2-methylpentenal.

The aqueous residue from the steam distillation had a *p*H of 10.3. The solution was adjusted to *p*H 8.0 with 0.1 *N* hydrochloric acid (11.8 ml. was required) and again subjected to steam distillation. An additional 300 ml. of distillate was collected and 13.3 ml. of 0.1 *N* hydrochloric acid solution was required to re-adjust the aqueous residue to *p*H 8. The solution was then evaporated to dryness on the steam-bath, the residue was dissolved in 5.0 ml. of water, and the *p*H was adjusted to 7.2. To this solution 10.0 ml. of ethanol and 0.50 g. of *p*-bromophenacyl bromide were added and the mixture refluxed for 3 hours. On cooling, an insoluble product formed. This material was digested with hot petroleum ether, and the insoluble portion crystallized from ethanol. The crystalline bis-ester of IX thus obtained melted at 138–141°.

The X-ray diffraction pattern and the melting point of this product were identical with the corresponding properties of an authentic sample of the bis-(*p*-bromophenacyl) ester of α,α'-dimethyl-β-hydroxyglutaric acid (see below); a mixed melting point was undepressed.

C_7-Hydroxylacetone B XII and α,α'-Dimethyl-β-hydroxyglutaric Acid (IX) by Peroxidation of III + IV.—The reaction with peroxytrifluoroacetic acid was carried out according to the procedure of Emmons[8] for the oxidation of ketones to esters. Trifluoroacetic anhydride (38 ml., 0.27 mole) was added dropwise with stirring to a solution of 8.5 g. of 90% hydrogen peroxide in 40 ml. of methylene chloride. The temperature of the mixture was maintained between 5 and 9° during the addition and afterwards. The solution of the oxidizing agent was then added dropwise to a stirred suspension of 170 g. of anhydrous disodium hydrogen phosphate in a solution of 13.5 g. (about 35 mM.) of III + IV (prepared from 15.75 g. of I) in 400 ml. of methylene chloride. During the addition the solution boiled gently. After the addition was complete, the mixture was heated under reflux for one hour and allowed to stand at room temperature overnight without stirring. The insoluble salts were filtered off and washed with three 100-ml. portions of methylene chloride. The combined methylene chloride solutions were extracted with two 20-ml. portions of a 10% sodium carbonate solution and then with 30 ml. of a saturated sodium chloride solution. The solution was dried over magnesium sulfate and concentrated to small volume. A small amount of crystalline material was filtered off with the aid of 10 ml. of a mixture of ether and petroleum ether. This crystalline material, m.p. 210–215°, probably consisted of oxidation products of dehydration product A and was therefore not further investigated. The residue (3.4 g.), obtained from the filtrate after removal of the solvents under reduced pressure, was dissolved in 100 ml. of 50% aqueous methanol. and the *p*H of the solution was adjusted to 12 with 5 *N* aqueous sodinm hydroxide. The solution was allowed to stand for 18 hours at room temperature and was then extracted with five 15-ml. portions of chloroform to remove neutral, colored impurities. The *p*H of the solution was adjusted to 1.5 with a 10% hydrochloric acid solution. The solution was allowed to stand for 6 hours and then extracted with five 30-ml. portions of chloroform. The combined chloroform solutions were washed with 5 ml. of a saturated sodium chloride solution, dried over magnesium sulfate, and the solvents removed under reduced pressure. The oily residue was allowed to stand at 0° for 18 hours in the presence of 10 ml. of ether and 5 ml. of petroleum ether. A small amount of crystalline material was obtained and identified as C_7-hydroxylactone B XII by comparison of the X-ray diffraction pattern with the sample of XII prepared from III as described below.

The insoluble salts filtered off from the reaction mixture were dissolved in 500 ml. of water, and the *p*H of the solution was adjusted to 12 by the addition of 2 *N* sodium hydroxide solution. Hydrolysis (of the acylal IV) was taking place as evidenced by a strong odor of propionaldehyde and a drifting toward lower *p*H. The *p*H was maintained at 11.5 for 24 hours by the occasional addition of the required amount of 2 *N* sodium hydroxide solution. The basic solution was extracted with three 50-ml. portions of chloroform and then adjusted to *p*H 2 by the addition of a cold 20% hydrochloric acid solution. The acidic components were extracted with five 75-ml. portions of ether. The aqueous solution was then saturated with sodium chloride and extracted with ether in the same manner. The combined ether extracts were washed with 25 ml. of a saturated sodium chloride solution, dried over magnesium sulfate and concentrated to small volume under reduced pressure. The crystalline product (IX, 1.22 g.) obtained was recrystallized from ether. Titration revealed two acidic groups with pK'_a values of 3.9 and 5.3, respectively, and an apparent molecular weight of 179 (calcd. for $C_7H_{12}O_5$, 176). The acid IX was optically inactive; $[\alpha]^{33}D$ −0.1°. The identity of this acid with an authentic sample of α,α'-dimethyl-β-hydroxyglutaric acid (see below) was established by a comparison of the X-ray diffraction patterns and the infrared spectra.

The bis-(*p*-bromophenacyl) ester prepared from the acid was found to be identical with the corresponding ester of the synthetic acid; X-ray diffraction patterns and infrared spectra were identical, and the mixed melting point (136°) was not depressed.

Anal. Calcd. for $C_{23}H_{22}O_7Br_2$: C, 48.44; H, 3.89; Br, 28.03. Found: C, 48.23; H, 4.08; Br, 28.23.

A further amount of 400 mg. of acid IX was isolated from the basic washes of the original methylene chloride reaction solution by acidification and extraction with ether. The total yield of acid IX amounted to 1.6 g. (24% based on I).

Periodate Titration of II.—Dihydroerythronolide (99.4 mg.) was suspended in 50 ml. of 0.1 *N* sodium hydroxide solution. The mixture was heated on the steam-bath for 15 minutes. The resulting solution was adjusted to *p*H 6.85 using 10% sulfuric acid solution; 12.5 ml. of 0.1 *N* sodium metaperiodate solution was added and the total volume was made up to 125 ml. Ten-milliliter aliquots were titrated with 0.01 *N* arsenite solution and compared with a blank. Three moles of periodate per mole of I was consumed in less than one-half hour. A fourth mole was consumed over a period of 24 hours.

Propionaldehyde and Acetic Acid by Periodate Oxidation of II.—Dihydroerythronolide (I, 0.92 g., purified by chromatography) was mixed with 40 ml. of 0.1 *N* aqueous sodium hydroxide, and the mixture was heated gently until solution was complete. The reaction mixture was then cooled and adjusted to *p*H 6.9 with 0.1 *N* aqueous sulfuric acid; 67 ml. of 0.1 *N* aqueous sodium metaperiodate was added, and the resulting solution was allowed to stand overnight at room temperature. A stream of nitrogen was then passed through the solution into 150 ml. of Brady reagent for 5 hours. The precipitate formed was removed by filtration and weighed 170 mg. (m.p. 140–150°). After recrystallization from ethanol, the product melted at 150–153° and its X-ray diffraction pattern was identical with that of an authentic sample of the 2,4-dinitrophenylhydrazone of propionaldehyde.

The aqueous reaction mixture, after treatment with nitrogen, was treated with 50 ml. of a saturated aqueous solution of barium hydroxide. The precipitated barium salts were removed by filtration, and the filtrate was acidified with aqueous 1 *N* sulfuric acid. The precipitated barium sulfate was removed by filtration and the filtrate steam distilled at constant volume; 300 ml. of distillate was collected. The distillate was titrated with 0.1 *N* aqueous sodium hydroxide to *p*H 9.0; 21.9 ml. of base (97.5% of theory) was consumed. The distillate was then evaporated to dryness on the steam-bath and the residue dissolved in 3.3 ml. of water. To this solution 6.5 ml. of ethanol and 0.65 g. of *p*-bromophenacyl bromide were added, and the mixture was heated under reflux for 1.5 hours. On cooling, a crystalline ester was obtained which on recrystallization

from petroleum ether melted at 81–83°. The X-ray diffraction pattern of this product was identical with the pattern of an authentic sample of the *p*-bromophenacyl ester of acetic acid.

C_9-Fragment III by Periodate Oxidation of II.—Dihydroerythronolide (I, 21 g.) was heated gently in 800 ml. of 0.5 *N* aqueous sodium hydroxide until the solid had completely dissolved (one hour). The solution was allowed to cool to room temperature and extracted with two 100-ml. portions of chloroform to remove any unhydrolyzed material. The *p*H of the solution was adjusted to 6.8 with cold 20% hydrochloric acid solution; 1.5 l. of 0.1 *N* sodium metaperiodate was added and the reaction mixture was allowed to stand at room temperature for one hour. A sample of the solution gave a positive test for periodate ion. The solution was extracted with five 500-ml. portions of chloroform, and the combined chloroform solutions were washed first with 100 ml. of a saturated sodium carbonate solution, with 50 ml. of a saturated sodium bisulfite solution, and finally with 75 ml. of a saturated sodium chloride solution. The chloroform solution was dried over magnesium sulfate and concentrated under reduced pressure leaving III as a sirupy residue, weighing 6.4 g. (74%). The infrared spectrum showed hydroxyl absorption, strong carbonyl absorption at 5.9 μ with shoulders at 5.95 and 6.1 μ. The ultraviolet spectrum showed a maximum at 2.28 mμ, ϵ 760, indicating the presence of about 10% of α,β-unsaturated aldehyde. The product III could not be distilled at 0.5 mm. without extensive decomposition.

Bis-(2,4-dinitrophenylhydrazone) of III.—A sample of (0.69 g.) III as prepared above was dissolved in 100 ml. of alcohol and 1.5 g. of 2,4-dinitrophenylhydrazine was added. The mixture was heated to boiling, 2 ml. of concentrated hydrochloric acid was added, and the solution was boiled for 15 minutes. Refrigeration gave 0.8 g. of product melting over a wide range at about 100°. This was chromatographed using 25 g. of silica and chloroform as the solvent. The product obtained from the first 120 ml. of effluent was recrystallized four times from acetone. The final melting point was 235–237°.

Anal. Calcd. for $C_{21}H_{22}N_8O_8$: C, 49.03; H, 4.32; N, 21.77. Calcd. for $C_{21}H_{24}N_8O_9$: C, 47.37; H, 4.57; N, 21.02. Found: C, 49.58; H, 5.63; N, 21.65.

On other samples the following values were found: C, 48.59, 49.31, 48.02, 49.71; H, 4.93, 4.77, 4.70, 4.60; N, 21.62, 21.19, 20.47, 20.49.

This compound had strong absorption in the ultraviolet at 367 mμ, ϵ 19,600. There was no absorption in the infrared spectrum in the hydroxyl region, but there was a strong band at 6.2 μ with a shoulder at 6.28 μ.

Acetyl-2,4-dimethylcyclopentadiene (XV) 2,4-Dinitrophenylhydrazone from III.—A sample (0.6 g.) of III as prepared above was dissolved in 60 ml. of 33% ethanol. The solution was adjusted to *p*H 12.0 with 5% sodium hydroxide solution. After the reaction mixture had stood at *p*H 12.0 for one-half hour, it was neutralized with sulfuric acid (10% by volume) and steam distilled until 250 ml. of distillate was collected. This was added to a solution of 0.2 g. of 2,4-dinitrophenylhydrazine in a mixture of 105 ml. of concentrated hydrochloric acid and 600 ml. of water. The dark red precipitate which formed on standing was filtered off. It weighed 0.48 g. and melted at 165–180° dec. Two recrystallizations from acetone gave an almost black product melting at 200° dec.

Anal. Calcd. for $C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.08; N, 17.71; mol. wt., 316. Found: C, 56.87; H, 5.30; N, 17.75; mol. wt., 321.

This hydrazone had maximum absorption in the ultraviolet at 402 mμ, ϵ 75,800.

***O*-Acetyl-C_7-lactone B XVI and C_7-Lactone B XII by Peroxidation[8] of III.**—Trifluoroacetic anhydride (19 ml., 0.135 mole) was added dropwise with stirring to a solution of 4.25 g. of 90% hydrogen peroxide in 20 ml. of cold methylene chloride. The temperature of the mixture was maintained between 5 and 9° during the addition and afterwards. The solution of the oxidizing agent was then added dropwise to a stirred suspension of 85 g. of anhydrous disodium hydrogen phosphate in a solution of 6.4 g. (0.37 mole) of II as prepared above in 100 ml. of methylene chloride. During the addition the solution boiled gently. In the course of the addition two 100-ml. portions of methylene chloride were added to facilitate stirring. After the addition was complete, the reaction mixture was heated under reflux for one hour and allowed to stand overnight without stirring at room temperature. The insoluble salts were filtered off and washed with three 100-ml. portions of methylene chloride. The combined methylene chloride solutions were extracted with two 25-ml. portions of 10% sodium bicarbonate solution; these basic extracts yielded no organic product. The methylene chloride solution was then washed with two 20-ml. portions of a saturated sodium chloride solution, dried over magnesium sulfate and concentrated to small volume. The oily residue obtained (3 g.) was neutral, and it gave no precipitate with Brady reagent. The infrared spectrum showed weak hydroxyl absorption and a broad carbonyl band; the ultraviolet spectrum was transparent between 220 and 400 mμ. Titration of a sample of the residue showed the absence of titratable groups, but slow hydrolysis was observed on exposure to alkali. The residue was taken up in 5 ml. of ether and 2 ml. of petroleum ether was added. The solution was allowed to stand at 0° for 12 hours; the long needles which formed were filtered off and dried thoroughly *in vacuo* at room temperature. Saponification followed by titration showed an acidic group(s) with a pK'_a of 4.64 and an apparent molecular weight of 99.5 (theory 93.1). Recrystallization from a mixture of ether and petroleum ether gave pure O-acetyl-C_7-lactone B XVI, m.p. 65–66°. The infrared spectrum of this material showed no hydroxyl absorption, but there was absorption in the carbonyl region between 5.65 and 5.88 μ.

Anal. Calcd. for $C_9H_{14}O_4$: C, 58.05; H, 7.58; CH_3CO–, 23.12; mol. wt., 186.2. Found: C, 58.24; H, 7.57; CH_3CO–, 23.34; mol. wt., 185.6 (X-ray crystallographic analysis).

The insoluble salts filtered off from the reaction mixture were dissolved in water, the *p*H of the solution was adjusted to *p*H 3.0 with 6 *N* sulfuric acid solution, and the solution was allowed to stand at room temperature for 18 hours to allow the lactonization of the presumed intermediate δ-O-acetyl-C_7-dihydroxyacid to C_7-hydroxylactone B to proceed. The solution was extracted with six 50-ml. portions of chloroform. The combined chloroform solutions were washed with 10 ml. of a saturated sodium chloride solution, dried over magnesium sulfate and concentrated under reduced pressure to small volume. The residue (1.1 g.) consisted of C_7-hydroxylactone B XII, probably contaminated with small amounts of acetic acid and/or trifluoroacetic acid and could not be obtained in crystalline form. It was converted to two crystalline products, XII and its hydrazide XVII, by treatment (a) with base and (b) with hydrazine, respectively.

(a) One-half of the above residue was dissolved in a mixture of 10 ml. of methanol and 10 ml. of water. The solution was adjusted to *p*H 12 and maintained at this *p*H with 1 *N* sodium hydroxide solution. After 3 days the solution had become brown and some of the colored impurities were extracted with four 10-ml. portions of chloroform. The solution was adjusted to *p*H 1.2, allowed to stand at room temperature for 4 hours and then extracted with three 30-ml. portions of chloroform. The combined chloroform extracts were washed with three 2-ml. portions of a saturated sodium chloride solution and dried over magnesium sulfate. The chloroform was removed under reduced pressure leaving a small amount of acidic material which could not be obtained in crystalline form. The aqueous solution was adjusted to *p*H 3.5 and extracted again with five 30-ml. portions of chloroform. The combined chloroform layers were washed and dried as before. The residue obtained yielded about 50 mg. of crystalline product XII melting at 92–94°. One recrystallization from a mixture of ether and petroleum ether raised the melting point to 93–94°. The infrared spectrum and the X-ray diffraction pattern were identical with those of analytically pure material prepared as described below under "Conversion of C_7-Hydrazide B XVII to C_7-Lactone B XII."

(b) The remaining half of the above residue was dissolved in 5 ml. of absolute methanol and heated on the steam-bath with 10 drops of anhydrous hydrazine for 18 hours. Removal of solvents under reduced pressure gave a residue which crystallized upon standing with a mixture of ethanol and ether. The X-ray diffraction pattern of this material XVII was identical with that of the samples described

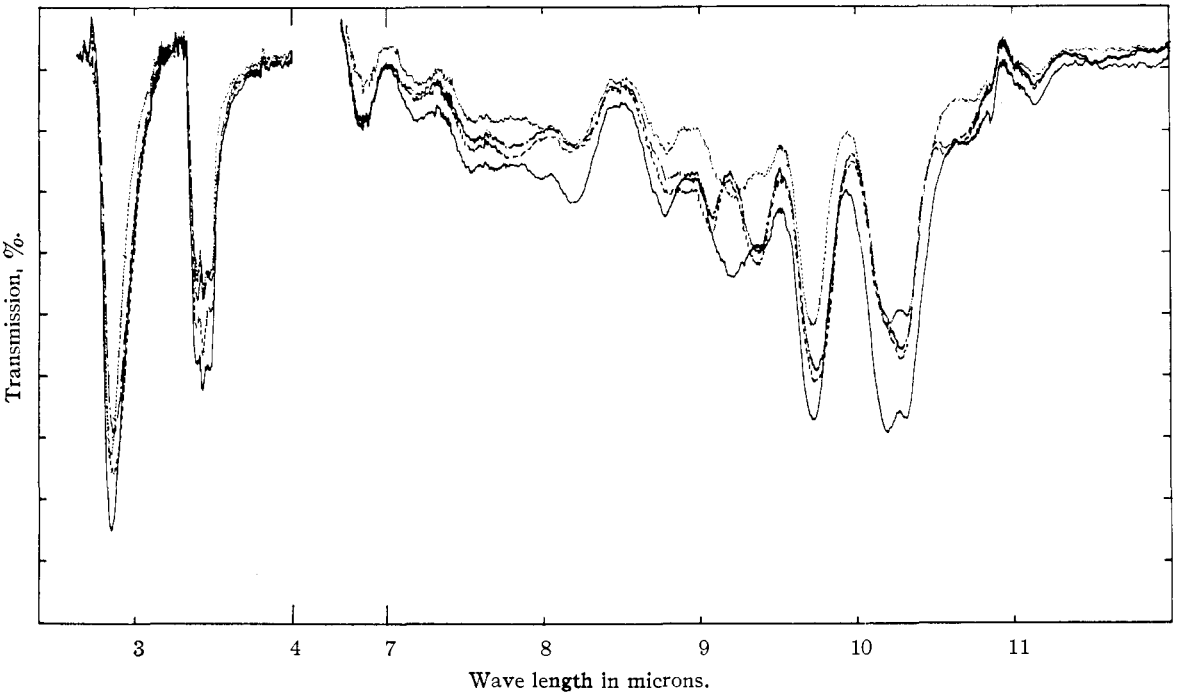


Fig. 7.—- - - - Infrared spectra in acetonitrile solution: . . . . ., *meso*-triol VIII; ———, synthetic *meso*-triol VIII; – – – –, *act*-triol XVIII; — · — · —, synthetic *rac*-triol XIX; cell path 0.1 mm., concentrations 5–6%.

below under "C_7-Hydrazide B XVII from O-Acetyl-C_7-lactone B XVI."

C_7-Hydrazide B XVII from O-Acetyllactone B XVI.—O-Acetyllactone B (550 mg.) was dissolved in 3 ml. of methanol and heated for 18 hours with 10 drops of anhydrous hydrazine in a closed vessel at 90°. The solvent and excess hydrazine were removed under reduced pressure, and the solid residue was crystallized from an ethanol–ether mixture to give 300 mg. of hydrazide melting at 145–151°. Recrystallization from the same solvent mixture raised the melting point to 152–155°. Titration in water revealed a group with a pK'_a of 3.4 with an apparent molecular weight of 180 (theory 176). The X-ray diffraction pattern and infrared spectrum differed from those of C_7-hydrazide A X.

Anal. Calcd. for $C_7H_{16}N_2O_3$: C, 47.73; H, 9.09; N, 15.91; C-CH_3 (2), 17.06. Found: C, 48.18; H, 9.18; N, 16.13; C-CH_3, 15.25; $[\alpha]^{32}D$ +6.7° (*c* 1, in methanol).

Conversion of C_7-Hydrazide B XVII to C_7-Lactone B XII.—C_7-Hydrazide B (70 mg.) was dissolved in 10 ml. of 1 *N* hydrochloric acid, and the acidic solution was stirred with 25 ml. of chloroform at room temperature for 18 hours. The layers were separated, and the acidic layer was extracted with three 12-ml. portions of chloroform. The combined chloroform extracts were washed with 3 ml. of 5% aqueous sodium carbonate, with 2 ml. of a saturated sodium chloride solution and dried over magnesium sulfate. After the removal of the chloroform under reduced pressure, the residue was allowed to crystallize from a mixture of ether and petroleum ether. The product obtained melted at 93–94°. The infrared spectrum showed hydroxyl absorption at 2.9 μ and lactone absorption at 5.8 μ; the ultraviolet spectrum was transparent between 220 and 400 mμ. Saponification followed by titration in water showed a group with a pK'_a of 4.53, apparent molecular weight 155 (theory 144). The X-ray diffraction pattern was different from that of C_7-lactone A VI.

Anal. Calcd. for $C_7H_{12}O_3$: C, 58.33; H, 8.33; C-CH_3 (2), 20.86. Found: C, 58.59; H, 8.51; C-CH_3, 15.15; $[\alpha]^{25}D$ +56.1° (*c* 1, in methanol).

C_7-Lactone B gave a negative iodoform test.

Active-2,4-dimethylpentane-1,3,5-triol (XVIII) by Reduction of C_7-Lactone B XII.—Lactone B (140 mg.) was dissolved in 17.5 ml. of anhydrous ether, and this solution was added dropwise to a stirred suspension of 400 mg. of lithium aluminum hydride in 70 ml. of anhydrous ether. The mixture was heated under reflux for one hour after which time the excess lithium aluminum hydride was decomposed by the addition of 0.4 ml. of water, 0.3 ml. of a 20% sodium hydroxide solution and finally 1.5 ml. of water. The solids were filtered off and washed three times with three 25-ml. portions of anhydrous ether. The combined ether layers were washed with 2 ml. of a saturated sodium chloride solution and dried over magnesium sulfate. Five milliliters of methylene chloride was added to the solution, and the solvents were removed under reduced pressure. The residue was dissolved in 3 ml. of anhydrous ether and allowed to stand at 0° for 3 days. The crystalline product (44 mg.) was filtered in a dry atmosphere and stored in a desiccator. The dry material melted at 54–56°; the refractive index of the melted material was $n^{25}D$ 1.4756. The infrared spectrum showed hydroxyl absorption at 2.9 μ and lactone absorption at 5.8 μ. The infrared spectra in acetonitrile and in chloroform solution of this triol and of the *meso*-triol VIII were virtually identical below 6 μ, but there were distinct differences in the region of 9–11 μ. The X-ray diffraction pattern differed from that of the *meso*-triol VIII.

Anal. Calcd. for $C_7H_{14}O_3$: C, 56.73; H, 10.88. Found: C, 57.00; H, 10.82; $[\alpha]^{25}D$ −14.0° (*c* 2, in methanol).

The infrared spectra of the *act*-triol XVIII in acetonitrile and in chloroform solution were *identical* with those of the synthetic *rac*-triol XIX (see below). The X-ray diffraction pattern of the *act*-triol differed from that of the *rac*-triol XIX (Fig. 7).

Synthetic Preparations. *rac*-Diethyl α,α'-Dimethyl-β-hydroxyglutarate (XX).—This ester was prepared according to the procedure of Reformatzki[5] using the improved technique of Newman.[40] From 180 g. (1 mole) of ethyl α-bromopropionate and 37 g. (0.5 mole) of ethyl formate there was obtained 40 g. (35% of theory) of the di-ester boiling at 115° (0.2 mm.), $n^{25}D$ 1.4385.

meso-α,α'-Dimethyl-β-hydroxyglutaric Acid (IX) from the *rac*-Di-ester XX.—The di-ester (103 g., 0.445 mole) was stirred magnetically under an atmosphere of nitrogen with a solution containing 40.5 g. of sodium hydroxide in 600 ml. of water. After the emulsion had clarified the solution was allowed to stand at room temperature for 18 hours and then at 60° for 4 hours. The solution was brought to *p*H 1.5 with cold 20% hydrochloric acid, and the glutaric acid was

(40) M. S. Newman and F. J. Evans, Jr., THIS JOURNAL, 77, 946 (1955).

extracted with three 250-ml. portions of ether. The aqueous solution was saturated with sodium chloride and again extracted with three 250-ml. portions of ether. The combined ether extracts were washed with 50 ml. of a saturated sodium chloride solution and dried over magnesium sulfate. The ether solution was concentrated to a volume of about 100 ml. and allowed to stand at room temperature for 3 days. The crystalline acid (15 g.) was filtered off and was used without further purification in subsequent preparations. The product melts at 85–90° with resolidification to melt again at 133–135° with gas evolution (anhydride formation).

O-Acetyl-α,α'-dimethyl-β-hydroxyglutaric Anhydride.[5]—*meso*-α,α'-Dimethyl-β-hydroxyglutaric acid (12 g., 0.068 mole) was suspended in 53 g. of acetyl chloride (0.68 mole), and the reaction mixture was swirled occasionally. The mixture was allowed to stand at room temperature for 24 hours; an equal volume of ether was added and the solution was filtered to remove a small amount of undissolved material. The crystalline product which formed on standing at room temperature for 3 hours was filtered off, washed with ether and dried. The filtrate was allowed to stand at 0° for 12 hours and yielded a second crop of crystals. The combined products weighed 5.5 g. and melted at 111.5°.

***meso*-2,4-Dimethylpentane-1,3,5-triol (VIII).** (a) **From *meso*-α,α'-Dimethyl-β-hydroxyglutaric Acid.**—The glutaric acid (7 g., 0.04 mole) was dissolved in 300 ml. of anhydrous ether and this solution was added dropwise to a stirred suspension of 6.8 g. (0.18 mole) of lithium aluminum hydride in 250 ml. of anhydrous ether. The reaction was carried out as described under "*meso*-Triol VIII by Reduction of C_7-Lactone A VI," using 6.5 ml. of water, 5.25 ml. of a 20% sodium hydroxide solution and 25.5 ml. of water. The residue (2.1 g.) obtained was distilled (b.p. 135° at 0.06 mm.), and the distillate was allowed to stand for 10 days at room temperature. The crystalline triol obtained melted at 75–78°; the refractive index of the melted material was $n^{25}D$ 1.4827.

Anal. Calcd. for $C_7H_{16}O_3$: C, 56.73; H, 10.88. Found: C, 56.88; H, 10.70.

(b) **From O-Acetyl-α,α'-dimethyl-β-hydroxyglutaric Anhydride.**—The glutaric anhydride (5 g., 0.029 mole) was reduced as described above with 11 g. (0.29 mole) of lithium aluminum hydride. The residue obtained crystallized prior to distillation and was identical in all respects with the *meso*-triol as obtained directly from the acid.

***rac*-2,4-Dimethylpentane-1,3,5-triol (XIX) by Reduction of Diethyl α,α'-Dimethyl-β-hydroxyglutarate (XX).**—The diethyl glutarate (32.5 g., 0.14 mole) was reduced with 24 g. (0.63 mole) of lithium aluminum hydride as described above. The residue (8 g.) was allowed to stand with 10 ml. of anhydrous ether at 0° for 2 days. The crystalline material (2.0 g.) was filtered off in a dry atmosphere, and the residue from the filtrate was distilled at 0.08 mm. (bath temperature 180°) to give 2 g. of a distillate from which a further amount (1 g.) of crystalline material was obtained by digestion with ether at 0°. Recrystallization of the crystalline material from ether gave pure triol XIX, m.p. 86–87°; the refractive index of the melted material was $n^{25}D$ 1.4752. The infrared spectrum in acetonitrile, or in chloroform solution, of the crystalline material and of the liquid distillate were identical indicating the *rac*-triol XX to be homogeneous and to contain little, if any, *meso*-triol VIII (Fig. 7).

Anal. Calcd. for $C_7H_{16}O_3$: C, 56.73; H, 10.88. Found: C, 56.57; H, 10.86.

The 1,5-bis-(*p*-toluenesulfonate) of *rac*-triol XIX after recrystallization from ether melted at 111°.

Anal. Calcd. for $C_{21}H_{18}S_2O_7$: C, 55.26; H, 6.18; S, 14.05. Found: C, 55.64; H, 6.21; S, 13.56.

Enanthic Acid by Peroxidation of Enanthaldehyde.—The oxidation was carried out according to the procedure used by Emmons and Lucas[8] for the oxidation of ketones to esters. Trifluoroacetic anhydride (25.4 ml., 0.18 mole) was added with stirring to 4.1 ml. of 90% hydrogen peroxide (0.15 mole) dissolved in 25 ml. of methylene chloride. The temperature of the mixture was maintained at 5 to 9° during the addition and afterwards. The solution of oxidizing reagent was then added dropwise to a stirred suspension of 85 g. of dry, finely powdered disodium hydrogen phosphate in a solution of 90 ml. of methylene chloride and 11.4 g. (0.1 mole) of *n*-heptaldehyde. After all the reagent had been added, the reaction mixture was heated under reflux for 30 minutes and allowed to stand at room temperature overnight. The salts were filtered off and washed with three 50-ml. portions of methylene chloride. The combined methylene chloride solutions were extracted with two 25-ml. portions of a 10% sodium carbonate solution and two 25-ml. portions of a 5% sodium bicarbonate solution. The combined basic extracts were acidified with concentrated hydrochloric acid (*p*H 2.7). The oily layer which formed was separated, and the aqueous layer was extracted with three 75-ml. portions of chloroform. The combined chloroform solutions were dried over magnesium sulfate, and the chloroform was removed under reduced pressure. The residue gave no precipitate with Brady reagent. Titration in 66% dimethylformamide showed a pK'_a of 7.67, apparent molecular weight 128; calculated 130. The infrared spectrum of the distilled product was identical with that of an authentic sample of *n*-heptanoic acid. The yield of *n*-heptanoic acid was 9 g. (70%).

INDIANAPOLIS, INDIANA

COMMUNICATIONS TO THE EDITOR

THE MECHANISM OF "ACTIVE SULFATE" FORMATION[1]

Sir:

The nucleotide-linked "active sulfate"[2,3,4] known to act as sulfate donor in the sulfurylation of phenols has been identified as 3′-phosphoadenosine-5′-phosphosulfate (PAPS).[5] Previous studies from this laboratory have shown that two heat-labile fractions are required for the formation of PAPS.[6] Evidence is here presented that enzyme I, ATP-sulfurylase, catalyzes the formation of adenosine-5′-phosphosulfate (APS) which is then converted into PAPS by enzyme II, adenosine phosphosulfate kinase (APS-kinase), as shown in the reaction sequence

$$\mathrm{ATP} + \mathrm{SO_4^=} \underset{}{\overset{\text{Enz I}}{\rightleftharpoons}} \mathrm{APS} + \mathrm{PP} \qquad (1)$$

$$\mathrm{APS} \xrightarrow[\mathrm{ATP}]{\text{Enz II}} \mathrm{PAPS} \qquad (2)$$

(1) We are indebted to the National Science Foundation for their support of this work.

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(6) L. G. Wilson and R. S. Bandurski, *Arch. Biochem. and Biophys.* **62**, 503 (1956).