that the material did not behave as a homogeneous substance and that the average molecular weight as computed by the Archibald[15] procedure was approximately 2000.

The amino acid content of Preparation C was investigated by the starch column technique of Moore and Stein[16]; the results obtained are summarized, together with other data, in Table IV. When it is compared with Preparation A2 in regard to amino acid content, it may be noted that Preparation C is considerably higher in histidine, lysine, arginine, valine and threonine. The preparation is essentially free from methionine and tyrosine.

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BERKELEY, CALIFORNIA RECEIVED NOVEMBER 2, 1951

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[15] W. J. Archibald, *J. Phys. Colloid Chem.*, **51**, 1204 (1947).

[16] S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO., INC.]

# Terramycin. II. Alkaline Degradation

BY R. PASTERNACK, A. BAVLEY, R. L. WAGNER, F. A. HOCHSTEIN, P. P. REGNA AND K. J. BRUNINGS

The alkaline degradation of the antibiotic terramycin produces ammonia, dimethylamine, carbon dioxide, acetic acid and a number of nitrogen-free compounds. Utilizing differences in acid strength, the following compounds have been isolated from the nitrogen-free fractions: (1) a strongly acidic compound $C_{13}H_{12}O_6$ which has been named terracinoic aicd, (2) 7-hydroxy-3-methylphthalide, (3) a phenolic compound, $C_{12}H_{10}O_3$ and (4) an as yet impure crystalline fraction with the acidity of an aliphatic carboxylic acid. Fractionation of the products of the caustic fusion of terramycin has yielded salicylic acid, *m*-hydroxybenzoic acid and succinic acid.

Terramycin,[1,2] the broad spectrum antibiotic isolated from culture broths of *Streptomyces rimosus*, is readily degraded by the action of alkali and of acid.[3] Alkaline treatment appears to result in the more drastic cleavage of the molecule and to yield products which are either identifiable as such or lend themselves well to structural determination. This paper describes in detail methods of carrying out aqueous alkaline degradations of terramycin and the identification of the products isolated thus far.

On boiling a 20% aqueous sodium hydroxide solution of terramycin, one mole each of ammonia and dimethylamine are evolved within 24 hours. When the hydrolysis is carried out in sodium carbonate, the evolution of the volatile bases is slow and incomplete. In acid hydrolysis, which will be the subject of a subsequent paper, dimethylamine appears to be more readily liberated than ammonia. Dimethylamine can also be cleaved selectively from the molecule by reduction in zinc and glacial acetic acid.[3]

Acetic acid (0.4 mole) and carbon dioxide (0.7 mole) have also been isolated from the degradation of terramycin in 20% aqueous sodium hydroxide.

When the alkaline hydrolysis is carried out in an inert atmosphere in the presence of zinc, a number of nitrogen-free products can be isolated after completion of the evolution of ammonia and dimethylamine. The method of separation takes advantage of the differences in the acid strengths of these degradation products and involves preliminary extraction of the acidified reaction mixture with ether, followed by successive extraction of this ether solution with *p*H 5.5 buffer and aqueous sodium bicarbonate. The ether solution remaining after these extractions contains several weakly acidic phenolic compounds.

The principal nitrogen-free product of the alkaline degradation can be isolated from the *p*H 5.5 buffer extract. Crystallization of the crude compound from ethyl acetate yields a strongly acidic white crystalline compound, melting at 233–234° with evolution of gas. This compound, which is optically inactive, has the molecular formula $C_{13}H_{12}O_6$ and has been named terracinoic acid. Terracinoic acid loses carbon dioxide slowly on heating in aqueous solution, a property which must be considered in crystallizing the compound from water. Complete decarboxylation is readily accomplished by heating in strong mineral acids to yield decarboxyterracinoic acid, $C_{12}H_{12}O_4$, m.p. 169–170°. A methyl ester of terracinoic acid forms very readily in methanolic hydrogen chloride and esterification occurs to a considerable extent when crystallization is attempted in alcohols.

The use of zinc in the alkaline degradation ensures a relatively smooth reaction and better yields but is not essential for the formation of terracinoic acid. Yields are not markedly affected by decreasing concentration of alkali and a yield of 30% has been obtained from a reaction run in 4% aqueous sodium hydroxide. However, no terracinoic acid has been isolated when the degradation is carried out in alcoholic sodium hydroxide.

From the ether solution remaining after the removal of terracinoic acid, a less acidic fraction can be extracted with aqueous sodium bicarbonate. No pure compound has been isolated from the bicarbonate extract which contains about 10% of the total weight of the alkaline degradation products. However, titration data on an impure crystalline product indicated $pK_a$ values of about 5.5 and 9.3. Removal of this fraction from the ether extract is essential to the successful recovery of subsequent fractions.

When the ether solution remaining after the *p*H 5.5 buffer and sodium bicarbonate extractions is

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[1] A. C. Finlay, G. L. Hobby, S. Y. P'an, P. P. Regna, J. B. Routien, D. B. Seeley, G. M. Shull, B. A. Sobin, I. A. Solomons, J. W. Vinson and J. H. Kane, *Science*, **111**, 85 (1950).

[2] P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings and W. A. Lazier, THIS JOURNAL, **73**, 4211 (1951).

[3] R. Pasternack, P. P. Regna, R. L. Wagner, A. Bavley, F. A. Hochstein, P. N. Gordon and K. J. Brunings, *ibid.*, **73**, 2400 (1951).

concentrated to a small volume and benzene added, a crystalline compound is precipitated. This phenolic substance isolated in about 2–3% yield can best be purified through the triacetate by reductive cleavage of the acetyl groups with lithium aluminum hydride. The pure compound, melting at 172–173° has a $pK_a$ value of 9.6 and three acetylatable hydroxyls. Its composition is best represented by the formula $C_{12}H_{12}O_3$.

Sublimation of the ether–benzene soluble fraction yields a phenolic lactone which has been shown to be 7-hydroxy-3-methylphthalide.[4] The use of zinc in the alkaline degradation is essential for the isolation of the phenolic compounds here described.

Caustic fusion of terramycin yields 11 mole per cent. salicylic acid, 5% *m*-hydroxybenzoic acid and 17% succinic acid. These acids were purified and separated by sublimation and countercurrent distribution between water and benzene–ethyl acetate.

## Experimental[5]

**Identification of Ammonia and Dimethylamine.**—9.139 g. of terramycin dihydrate was boiled under efficient reflux for 22 hours with 150 ml. of 20% sodium hydroxide solution. The volatile bases were absorbed in an excess of standard hydrochloric acid. Back titration of an aliquot showed that 1.87 equivalents of bases had been liberated. Ammonia was determined on an aliquot by Nessler's method; it accounted for one-half of the total base. The remaining hydrochloric acid solution was evaporated to dryness and the dry salts extracted with chloroform. The insoluble salt was identified as ammonium chloride by analysis and refractive index.

*Anal.* Calcd. for $NH_4Cl$: N, 26.19; Cl, 66.36. Found: N, 26.09; Cl, 66.22; $n^{25}$ 1.638; $n^{25}{}_D$ of an authentic sample, 1.639.

Identification was confirmed through the preparation of ammonium chloroplatinate.

*Anal.* Calcd. for $(NH_4)_2PtCl_6$: N, 6.31; Pt, 43.97. Found: N, 6.38; Pt, 42.88.

The chloroform soluble salt was identified as dimethylamine hydrochloride, by conversion to N,N-dimethyl-*p*-toluenesulfonamide and to dimethylamine picrate. The sulfonamide prepared in the usual manner and recrystallized from 80–90° ligroin melted at 80–81°. A mixed melting point with an authentic sample showed no depression.

*Anal.* Calcd. for $C_9H_{13}NO_2S$: C, 54.25; H, 6.58; N, 7.03. Found: C, 54.24; H, 6.71; N, 6.95.

The picrate prepared in the usual manner and recrystallized from hot water melted at 158–160°, not depressed when mixed with an authentic sample.

**Terracinoic Acid.**—A stirred solution of 100 g. (0.2 mole) of terramycin dihydrate in 1000 ml. of 25% (weight/volume) sodium hydroxide containing 300 g. of finely granulated zinc was heated under reflux in a nitrogen atmosphere for 24 hours. After cooling in an ice-bath, the alkaline solution was decanted from the zinc, and slowly added to a mixture of 500 g. of sulfuric acid and 2 kilos of cracked ice overlayed with 500 ml. of ether. The aqueous layer was then extracted with five more 300-ml. portions of ether, and the combined ether solutions were extracted with six 300-ml. portions of 1.0 *M* phosphate buffer, *p*H 5.5. The buffer solution, which contains all of the terracinoic acid, was acidified with 200 ml. of 85% phosphoric acid and subsequently extracted with four 300-ml. portions of ether. Evaporation of the ether yielded 27 g. of terracinoic acid. The crude product, after washing with cold ethyl acetate to remove colored by-products, weighed 21 g. and melted at 230–232°. A further 3–4 g. was obtained from the ethyl acetate wash; yield 46%. Analytically pure material can be obtained by recrystallization from ethyl acetate or acetone–petroleum ether. The pure compound melts at 233–234° with decomposition, when placed in a bath at 220°, temperature rising 2° per minute.

*Anal.* Calcd. for $C_{13}H_{12}O_6$: C, 59.09; H, 4.58; mol. wt., 264. Found: C, 59.17, 59.09; H, 4.40, 4.74; mol. wt. (ebul. in acetone), 275 ± 20.

An electrometric titration with 0.1 *N* NaOH was plotted and from the three breaks in the titration curve $pK_a$ values of 2.6, 4.7 and 9.5 were calculated. The equivalent weight by titration is 265. Characteristic for terracinoic acid is the development of a strong magenta color with alcoholic or aqueous ferric chloride.

**Monomethyl Ester of Terracinoic Acid.**—Terracinoic acid, 5.3 g., dissolved in 50 ml. of anhydrous methanol was added to 50 ml. of 10% methanolic hydrochloric acid at room temperature. A crystalline precipitate started to separate within 30 minutes. After 24 hours the product was filtered, washed with methanol, and dried at 65° to yield 4.2 g. (75%) of analytically pure terracinoic acid methyl ester, m.p. 217–218°.

*Anal.* Calcd. for $C_{14}H_{14}O_6$: C, 60.43; H, 5.07; methoxyl, 11.12. Found: C, 60.32; H, 4.96; methoxyl (Zeisel), 11.17 (saponification), 11.26.

**Decarboxyterracinoic Acid.**—Five and two-tenths grams (0.02 mole) of terracinoic acid was heated under reflux with 75 ml. of 85% phosphoric acid in a slow stream of nitrogen. The effluent gases were passed through a 2% barium hydroxide solution. Decarboxylation, as determined from the weight of barium carbonate precipitated, amounted to 94 mole per cent. in 7 hours. Upon the addition of 225 ml. of water, crude decarboxyterracinoic acid separated overnight. After one recrystallization from water with Norite decolorization, the colorless crystals melted at 168–169°; yield 3.2 g. (74%). Three additional crystallizations from water raised the m.p. to 169–170°. Decarboxyterracinoic acid does not give a color reaction with ferric chloride.

*Anal.* Calcd. for $C_{12}H_{12}O_4$: C, 65.44; H, 5.47. Found: C, 65.41; H, 5.61.

**Bicarbonate-Soluble Fraction.**—The ether solution remaining after extraction with phosphate buffer was extracted with nine 250-ml. portions of 2% sodium bicarbonate. The bicarbonate extract was acidified, and re-extracted with ether to yield 7.2 g. of crude viscous tar. Sublimation at 220°, 0.05 mm., followed by crystallization from water yielded 0.5 g. of crystalline product, m.p. 104–108°. The titration curve of this crude product shows breaks corresponding to $pK_a$ 5.5 and 9.3, equivalent weight 262.

**Phenolic Compound $C_{12}H_{12}O_3$.**—The ether solution, after extraction with bicarbonate, was dried over sodium sulfate, and concentrated *in vacuo* to 20 ml. On standing overnight, a dark crystalline mass separated; precipitation was completed by the addition of 20 ml. of benzene. The crude product 3–4 g., obtained in this way, may be best purified through the triacetate. Two and one-half grams of crude compound was dissolved in 5 ml. of pyridine and 4 ml. of acetic anhydride. After 6 hours at room temperature, the reaction mixture was quenched in water, dried *in vacuo*, and extracted in a Soxhlet with cyclohexane. Two recrystallizations from cyclohexane, followed by recrystallization from benzene–ligroin, yielded 1.3 g. pure triacetate, m.p. 148.7–149.4°.

*Anal.* Calcd. for $C_{18}H_{18}O_6$: C, 65.45; H, 5.47; acetyl, 39.2; mol. wt., 330. Found: C, 65.25; H, 5.45; acetyl, 39.9; mol. wt. (Rast), 320.

The free phenol can be regenerated by hydrolysis in acid or alkali. However, the best product was obtained through reductive cleavage of the acetyl groups with lithium aluminum hydride.[6] Hydrolysis of 0.5 g. of triacetate in this manner yielded 0.13 g. of $C_{12}H_{12}O_3$, m.p. 172.4–173°.

*Anal.* Calcd. for $C_{12}H_{12}O_3$: C, 70.65; H, 5.97. Found: C, 70.47; H, 6.17.

The mother liquors yielded 0.14 g. of slightly less pure product. The titration curve of this compound showed a single break corresponding to $pK_a$ 7.6. It gives a green ferric chloride test and readily reduces both Fehling solution and ammoniacal silver nitrate.

**7-Hydroxy-3-methylphthalide.**—The benzene–ether mother liquor from the precipitation of the $C_{12}H_{12}O_3$ was evaporated to dryness, and the dark viscous residue evaporatively distilled at 140° (0.05 mm.) to yield a colorless viscous oil, which crystallized readily on exposure to moist air, or in contact with water. Crystallization from water, and from

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(4) F. A. Hochstein and R. Pasternack, THIS JOURNAL, **73**, 5008 (1951).

(5) All melting points are corrected.

(6) R. Nystrom and W. G. Brown, THIS JOURNAL, **69**, 1197 (1947).

water–methanol, yielded 0.5–1.0 g. of 7-hydroxy-3-methylphthalide as the monohydrate, $C_9H_8O_3 \cdot H_2O$, m.p. 109–110°.[3]

**Carbon Dioxide and Acetic Acid.**—Ten grams of terramycin dihydrate (0.0202 mole) was dissolved in 100 ml. of 25% sodium hydroxide, free of carbonate, and heated under reflux in a nitrogen atmosphere with 30 g. of acid washed granulated zinc for 22 hours.

The solution was decanted from the zinc, acidified to *p*H 1 with sulfuric acid, and again heated under reflux in a stream of nitrogen for 2 hours. Carbon dioxide was collected as barium carbonate (2.80 g., 0.0142 mole).

The acid solution was distilled to small volume, diluted with water and redistilled to yield 0.0078 mole of acetic acid, which was identified microscopically[7] as its sodium uranyl salt, and as its silver salt.

**Alkali Fusion of Terramycin.**—Terramycin base (25 g., 0.05 mole) was added portionwise over one hour to a melt of 120 g. of potassium hydroxide and 60 g. of sodium hydroxide at 200–210°. After 2 hours further heating, the melt was cooled, dissolved in water, acidified, and extracted with three 100-ml. portions of ethyl acetate. The ethyl acetate extract was evaporated to dryness and the tarry product sublimed at 200° (0.1 mm.) to yield 3.6 g. of oily crystals. Direct crystallization from water, followed by recrystallization, yielded 0.66 g. of salicylic acid, m.p. 158–159°, mixed melting point with an authentic sample not depressed. The identification was confirmed by the preparation of the acetyl derivative, m.p. 134–135°.

The aqueous mother liquors from the crystallization of the salicylic acid were subjected to a 12-plate countercurrent extraction *vs.* 1:1 ethyl acetate–benzene. Tubes 1 to 5 were fractionally recrystallized from water to yield a further 0.10 g. of salicylic acid (total yield, 0.76 g., 11%) and 0.35 g. (5%) of *m*-hydroxybenzoic acid, m.p. 199.5–201°, mixed m.p. with an authentic sample not depressed.

*Anal.* Calcd. for $C_7H_6O_3$: C, 60.90; H, 4.47; mol. wt., 138. Found: C, 60.81; H, 4.71; mol. wt. (Rast), 135.

Tubes 8 to 12 yielded on sublimation, 1.0 g. (17%) of succinic acid and succinic anhydride, which was dissolved in water, and resublimed at 0.05 mm. to yield succinic acid, m.p. 186–187°, mixed m.p. with an authentic sample not depressed.

*Anal.* Calcd. for $C_4H_6O_4$: C, 40.65; H, 5.13; mol. wt., 118. Found: C, 40.84; H, 5.38; mol. wt. (Rast), 123.

The succinic acid was further characterized by conversion to succinic anhydride, m.p. 118–119°, mixed m.p. with an authentic sample of m.p. 119–120°, not depressed.

**Acknowledgment.**—We are indebted to Dr. J. Means for the microanalyses.

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[7] E. M. Chamot and C. M. Mason, "Handbook of Chemical Microscopy," John Wiley and Sons, Inc., New York, N. Y., 1939, p. 389.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO., INC.]

# Terramycin. III. Structure of Terracinoic Acid, an Alkaline Degradation Product[1]

BY R. PASTERNACK, L. H. CONOVER, A. BAVLEY, F. A. HOCHSTEIN, G. B. HESS AND K. J. BRUNINGS

Terracinoic acid, $C_{13}H_{12}O_6$, a product of the alkaline degradation of the antibiotic terramycin, is shown to have the structure 4-carboxy-5-hydroxy-3-methylindanone-2-acetic acid. The principal features of the proof of structure are: (1) determination of the carbon skeleton by caustic fusion and hydrogenation, (2) proof of the presence of a $\gamma$-keto acid system by pyridazone formation and decarboxylation of 2-bromoterracinoic acid by dehydrobromination, and (3) determination of the orientation of the functional groups by comparative studies of acid strengths and of ultraviolet and infrared spectra of terracinoic acid and its derivatives. The basic structure was checked by showing that the ultraviolet spectrum of 5-hydroxyindanone is practically identical with that of decarboxyterracinoic acid.

Terracinoic acid,[2,3] $C_{13}H_{12}O_6$, is the principal nitrogen-free product formed by the aqueous alkali degradation of the antibiotic terramycin. In this paper, experimental evidence is presented for assigning to terracinoic acid the structural formula 4-carboxy-5-hydroxy-3-methylindanone-2-acetic acid (I).

Terracinoic acid is an optically inactive, colorless, tribasic acid melting at 233–234°. It gives a magenta color with ferric chloride, and a positive Millon test. Fehling and Tollens tests are negative. The presence of a benzene ring in the structure of terracinoic acid is suggested by the isolation of *m*-ethylphenol and of related carboxylic acids from the products of alkali fusion. Confirmation that the benzene ring is present as such in terracinoic acid is provided by nitration to yield a mononitro derivative, m.p. 201–202°, which can be reduced to an aromatic amine.

The hydrogenation of terracinoic acid over palladium and glacial acetic acid results in the absorption of 5.6 to 5.9 moles of hydrogen to yield a saturated dicarboxylic acid, $C_{13}H_{20}O_4$, m.p. 149.5–150.5°. The formula of this saturated compound is consistent with a bicyclic system and thus it may be concluded that terracinoic acid contains a benzene ring fused to a carbocyclic system.

The presence of an ethylenic double bond in terracinoic acid is not indicated by its behavior toward permanganate oxidation and bromination. Terracinoic acid reacts slowly with alkaline permanganate at room temperature to yield 2-hydroxyterracinoic acid (VII), $C_{13}H_{12}O_7$, m.p. 244–245°. On warming, a more vigorous reaction takes place resulting in drastic degradation of the molecule. Acetic acid (0.6 mole) has been isolated from this reaction, establishing the presence of a C-methyl group.

Terracinoic acid reacts readily with bromine in cold glacial acetic acid by substitution to form 2-bromoterracinoic acid (VI), $C_{13}H_{11}BrO_6$, m.p. 280–281° (dec.). The bromine in this compound is labile and may be replaced with a hydroxyl group by reaction in aqueous sodium acetate to yield the above-mentioned 2-hydroxyterracinoic acid. The 2-bromoterracinoic acid undergoes dehydrobromination and loss of a carboxyl group by the action of aqueous alkali to form compound VIII, $C_{12}H_{10}O_4$, m.p. 230.5–231°. In warm aqueous solution, the bromination of terracinoic acid results in decarboxylation and the formation of 4,6-dibromodecarboxyterracinoic acid, m.p. 169–170°. The bromine in this compound is not labile

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[1] Presented before the 12th International Congress of Pure and Applied Chemistry, September, 1951, in New York, N. Y.

[2] R. Pasternack and collaborators, THIS JOURNAL, **73**, 2400 (1951).

[3] R. Pasternack, A. Bavley, R. L. Wagner, F. A. Hochstein, P. P. Regna and K. J. Brunings, *ibid.*, **74**, 1926 (1952).