neutralized with aqueous sodium bicarbonate, the solvent was removed under reduced pressure, and the residue was extracted with methylene chloride. The extract was washed with water and was dried over anhydrous sodium sulfate. The yellow sirupy residue was chromatographed on 7.0 g. of alumina, first by gradient elution, using 100 ml. of benzene as the donor and 350 ml. of 1:9 benzene–petroleum ether as the recipient solvent, and subsequently by 100 ml. of benzene. The fractions from 310 ml. to 360 ml. of eluent contained a material which showed neither O–H stretching nor C=O stretching bands in the infrared spectrum, possibly unchanged 3,16α-dimethoxyestra-1,3,5(10)-trien-17-one ethylenehemithioketal. The peak from 420 ml. to 550 ml. gave 0.030 g. (23% yield from **9**) of a keto-compound identical with the product (B). Recrystallization from methanol afforded prisms of 3,16α-dimethoxyestra-1,3,5(10)-trien-17-one (**10**), m.p. 96–97.5°, λ_{max} 5.73 μ.

Anal. Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 75.98; H, 8.14.

3,16α-Dimethoxyestra-1,3,5(10)-trien-17β-ol (**11a**) **and 17α-ol** (**11b**).—A solution of 0.030 g. of ketone **10** in methanol was treated with 0.010 g. of sodium borohydride at ice–salt bath temperature for 30 min., and at 24° for another 30 min. After addition of a small amount of acetic acid, the solvent was flash-evaporated. The residue was mixed with water and extracted with methylene chloride. The usual work-up provided 0.027 g. of a sirup, which was chromatographed on 7.0 g. of alumina. The first peak, eluted with 1:10⁴ ethanol–benzene, gave 0.008 g. (27% yield) of crystals, which were recrystallized from 80% aqueous methanol, affording prisms of the 17α-ol **11b**, m.p. 116–117.5°, λ_{max} 2.80 μ (somewhat broad).

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.42; H, 8.89.

The second peak, eluted with 1:200 ethanol–benzene, gave 0.010 g. (33%) yield of crystals, which were recrystallized from 80% aqueous methanol, giving small plates of the 17β-ol **11a**, m.p. 139–140.5°; λ_{max} 278 mμ (ε 2030), 287 mμ (ε 1790); λ_{max} 2.75, 2.85 (broad) μ.

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.82; H, 8.76.

Acetylation of **11a** with acetic anhydride–pyridine afforded prisms of **3,16α-dimethoxy-17β-acetoxyestra-1,3,5(10)-triene**, m.p. 119–120°.

Anal. Calcd. for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.83; H, 8.17.

Methylation of Estriol 3-Methyl Ether to 3,16α-Dimethoxyestra-1,3,5(10)-triene-17β-ol (**11a**) **and 3,17β-Dimethoxyestra-1,3,5(10)-triene-16α-ol** (**4a**).—A suspension of 0.050 g. (0.15 mmole) of estriol 3-methyl ether, m.p. 160–161°, in 10 ml. of methylene chloride was methylated with 2.4 mmoles of DM in 8 ml. of methylene chloride and 0.18 ml. of boron trifluoride catalyst solution, as described under **3**. The sirupy product (0.063 g.) was chromatographed on 15.0 g. of alumina. Fractions eluted with benzene afforded 0.020 g. of a crystalline product which showed no O–H stretching band in the infrared region, probably estriol trimethyl ether. Fractions eluted with 1:200 ethanol–benzene gave 0.025 g. of a mixture of isomeric estriol dimethyl ethers, which was rechromatographed on 15.0 g. of alumina. The middle portions of the first peak, eluted with 1:500 ethanol–benzene, contained 3,17β-dimethoxyestra-1,3,5(10)-trien-16α-ol (**4a**); and the second peak, eluted with 1:500 ethanol–benzene, afforded the 3,16α-isomer **11a**. The identity of both isomers was confirmed by direct comparison with the specimens of unequivocal structures **4a** obtained from diol **7a**, and **11a** obtained from ketone **10**. The ratio of the amount of **4a** to **11a** was approximately 1:2. The final washing of the first chromatography column with 50 ml. of 1:1 ethanol–benzene contained 0.005 g. of recovered estriol 3-methyl ether.

Methyl (Bornyl-2′,3′,4′-tri-O-acetyl-β-D-glucopyranosid)-uronate.—Methylation of 0.70 g. of bornyl β-D-glucopyranosiduronic acid[26] with 40 mmoles of DM in dichloromethane–methanol gave 0.77 g. of a sirupy methyl ester which was acetylated with acetic anhydride–pyridine without further purification. Evaporative removal of the solvents from the reaction mixture gave a sirupy product which solidified on addition of water. Drying of the solid and recrystallization from *n*-hexane afforded 0.60 g. of colorless prisms of the methyl ester triacetate, m.p. 138–142° (71% yield). The analytical sample melted at 139–142°, $\alpha^{19}D$ −41.5° (*c* 2.0 in $CHCl_3$).

Anal. Calcd. for $C_{23}H_{34}O_{10}$: C, 58.71; H, 7.28. Found: C, 58.96; H, 7.23.

Nuclear magnetic resonance (**n.m.r.**) **spectra** were determined with an A-60 analytical n.m.r. spectrometer of Varian Associates, except for the insert, Fig. 2, upper curve, which was determined with an HR-60 n.m.r. spectrometer of Varian Associates. We wish to thank Dr. James N. Shoolery for his kind coöperation in determining the spectra, and for his helpful discussion.

(26) Commercial borneol glucuronic acid of Sigma Chemical Co., St. Louis, Mo., m.p. 156–158.5°, $\alpha^{20}D$ −59.5° (*c* 2.0 in H_2O).

[CONTRIBUTION NO. 1680 FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONN.]

The Structure of Serratamolide[1–3]

BY HARRY H. WASSERMAN, JANIS J. KEGGI AND JAMES E. MCKEON

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Serratamolide, $C_{26}H_{46}O_5N_2$, has been isolated from cultures of *Serratia* and has been shown to have structure II. Mild hydrolysis leads to serratamic acid (I). Formation of the following derivatives is described: diacetylserratamolide, the ditetrahydropyranyl ether, ditosylserratamolide and the ditrityl ether. Reduction of the free hydroxyl groups by the sequence –OH → –O–Mesyl → –Br → –H followed by hydrolysis with HCl yields alanine as well as 3-hydroxydecanoic acid.

During studies[4a,b] on the metabolic products from *Serratia marcescens*, strain HY-3[5a] and its mutant strains SM 9-3-3[5b] and P-1,[5c] we have isolated a neutral colorless compound, melting at 159–160°, which we have named serratamolide. This material was found on working up the mother liquors in the purification of prodigiosin and its C_{10}-precursor, and was first obtained from methylene chloride extracts of SM 9-3-3 liquid cultures. Subsequently it was isolated in greatly improved yield from HY-3, SM 9-3-3 or P-1, grown on glycerol–peptone agar medium.

(1) For a preliminary account of this work, see H. H. Wasserman, J. J. Keggi and J. E. McKeon, *J. Am. Chem. Soc.*, **83**, 4107 (1961).

(2) This work was supported in part by Grant P64 from the American Cancer Society and by Grant E-1729 from the Division of Research Grants, National Institute of Health, U. S. Public Health Service.

(3) Taken from the doctoral theses of J. J. Keggi (1962) and J. E. McKeon (1961), Yale University.

(4) (a) H. H. Wasserman, J. E. McKeon, L. Smith and P. Forgione, *J. Am. Chem. Soc.*, **82**, 506 (1960); (b) H. H. Wasserman, J. E. McKeon and U. V. Santer, *Biochem. and Biophys. Res. Comm.*, **3**, 146 (1960).

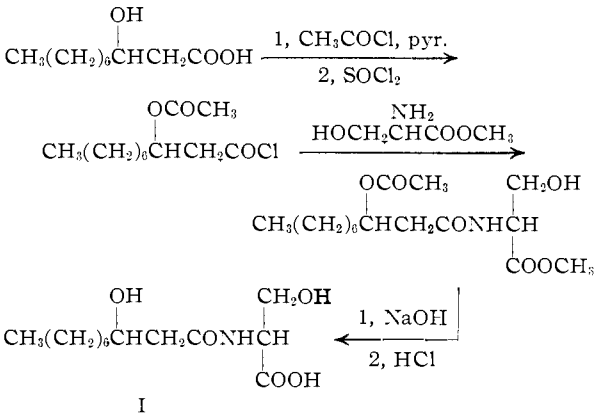
(5) (a) Strain HY-3 was provided by Dr. Mary I. Bunting; see E. L. Labrums and M. I. Bunting, *J. Bacteriol.*, **65**, 394 (1953); (b) U. V. Santer, Ph.D. Dissertation, Yale University, 1958; (c) M. T. M. Rizki, *Proc. Nat. Acad. Sci.*, **40**, 1057 (1954).

Serratamolide is optically active and contains no amino nitrogen (Van Slyke). The analytical and molecular weight data are consistent with a molecular formula $C_{26}H_{46}O_8N_2$, containing two C-methyl groups (Kuhn–Roth) and no methoxyl (Zeisel). No absorption in the ultraviolet above 200 mμ was found except for end-absorption above 250 mμ, and the infrared spectrum (KBr) shows bands at 5.80, 6.05, 6.46 and 3.01 μ.

These data suggest that serratamolide may be the same material as two other neutral, colorless products reported to have been obtained from *Serratia*. In one report, Castro and co-workers describe the isolation from cultures of *Serratia marcescens* of an amide, m.p. 154.3–156°[6] (infrared bands in mineral oil at 5.81, 6.04, 6.46 and 3.02 μ) to which a molecular formula $C_{24}H_{42-44}O_7N_2$ was assigned, while Efimenko, Kuznetsova and Yakimov[7] report the isolation from *B. prodigiosum* of a colorless material, m.p. 153°, which was considered to be $C_{34}H_{62}O_{10}N_2$.

Serratamolide contains two free, reactive hydroxyl groups, as shown by the ready formation of a number of characteristic derivatives: a diacetyl derivative, m.p. 222.5–223°(dec.); a di-tetrahydropyranyl derivative (mixture of isomers), m.p. 140–145°; an unstable ditosyl derivative, m.p. 150° dec.; and a dityryl ether, m.p. 186–187.5°. The analytical and molecular weight data on these derivatives are in good agreement with the assignment of the molecular formula $C_{26}H_{46}O_8N_2$ to the parent compound.

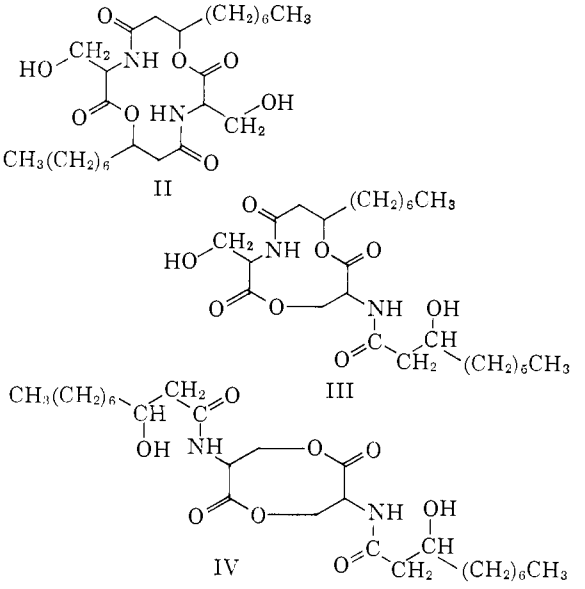
Mild hydrolysis of serratamolide in 1 *N* sodium hydroxide at room temperature yielded an acid, m.p. 137–138° dec., in 88% yield based on a ratio of two acid residues to one serratamolide molecule. This acid proved to be identical in melting point, mixture melting point and infrared spectra with serratamic acid, recently isolated by Cartwright[8] from several strains of organisms of the *Serratia* group. Cartwright assigned structure I to serratamic acid on the basis of the following experiments: (a) hydrolysis of I to L-serine and D-3-hydroxydecanoic acid. The latter was characterized by comparison with authentic D-3-hydroxydecanoic acid in the form of the *p*-bromophenacyl ester and the cinchonidine salt; (b) synthesis of serratamic acid by the recombination of L-serine and D-3-hydroxydecanoic acid in a sequence of steps, without isolation of intermediates, as



I

As would be expected from Cartwright's results, we have found that more vigorous hydrolysis of serratamolide in hot concentrated hydrochloric acid yielded serine, identified by ion exchange chromatography,[9] and an oily, acid-insoluble component which was shown to be 3-hydroxydecanoic acid by comparison of its ethyl ester with synthetic ethyl 3-hydroxydecanoate.

The generation of two molecules of the hydroxy acid I by hydrolysis of the neutral serratamolide must involve the opening of a dilactone such as II, III or IV, and the following structural work was directed at distinguishing among these most reasonable possibilities.



The infrared spectrum of serratamolide can be reconciled with all of the three structures, the peak at 5.79μ being due to the lactone carbonyl in a large ring and the bands at 6.05 and 6.46μ being characteristic of amide I and amide II absorption.[10] Though structure III would be expected to have a more complex spectrum than the symmetrical alternatives, it is not possible to rule out conclusively this possibility on the basis of infrared evidence alone.

The fact that the dityryl ether of serratamolide can be prepared quite readily strongly suggests that both of the free hydroxyl groups are primary. However, this cannot be considered unequivocal evidence in favor of structure II since secondary alcohols may also form trityl derivatives under

(6) A. J. Castro, A. H. Corwin, F. J. Waxham and A. L. Beilby, *J. Org. Chem.*, **24**, 455 (1959). A sample of this amide furnished by Dr. Castro has an infrared spectrum (KBr) superimposable upon that of serratamolide.

(7) O. M. Efimenko, G. A. Kuznetsova and P. A. Yakimov, *Biokhim.*, **21**, 416 (1956) (English translation, p. 419).

(8) N. J. Cartwright, *Biochem. J.*, **60**, 238 (1955); **67**, 663 (1957). We wish to thank Dr. Cartwright for sending us a sample of serratamic acid.

(9) Beckman/Spinco model 120 amino acid analyzer. The help of Dr. L. Wofsy and Miss M. Cherry in performing the amino acid assays is gratefully acknowledged.

(10) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Second edition, John Wiley and Sons, Inc., New York, N. Y., 1958, p. 217.

stronger conditions.[11] We therefore sought to distinguish between the structures under consideration by masking the free hydroxyl groups in serratamolide through the preparation of suitable hydroxyl derivatives, hydrolyzing the protected product, and identifying the resulting fragments.

Hydrolysis of the acetyl derivative in base was found to be an unpromising approach since this treatment led to the loss of the protecting groups and the formation of serratamic acid. Use of the di-tetrahydropyranyl derivative was also unprofitable since this derivative was inordinately resistant to the action of base—in fact, so inert that it was not possible to open the lactone ring without using such vigorous conditions that degradation ensued. Under milder acidic conditions the di-tetrahydropyranyl ether was smoothly reconverted to serratamolide.

A more fruitful approach was found in work directed at the reduction of the two free hydroxyl groups. Initial experiments were aimed at converting the tosylate to a diiodo compound for reductive dehalogenation in a further step. Although this approach was unsuccessful due to the instability of the diiodo derivative, the following modified sequence of reactions was found to be effective: (i) Treatment of serratamolide with methanesulfonyl chloride in pyridine at 0° gave the unstable dimethanesulfonyl derivative, m.p. 125–127° dec., which was rapidly converted with lithium bromide in refluxing methyl ethyl ketone to the neutral, moderately stable dibromo derivative, m.p. 170° dec. (ii) Hydrogenation of the dibromo derivative in ethanol or methanol in the presence of palladium and a small amount of aqueous base yielded a debrominated product (not isolated) which was immediately hydrolyzed with hot concentrated hydrochloric acid, producing a mixture of acids. As a result of this sequence of changes (–OH → –O-Mesyl → –Br → –H), II would yield alanine and 3-hydroxydecanoic acid, III would yield serine and decanoic acid, while IV would give rise to all four of the above acids.

Examination of the aqueous acidic hydrolysate, obtained as described above, by both paper chromatography and ion exchange chromatography[9] showed that alanine (67%) was the only amino acid present in more than trace amounts. (A control hydrolysis of serratamolide under the same conditions gave almost complete recovery of serine (91%) and no other amino acids.) The other acidic product of the stepwise reduction and hydrolysis was methylated with diazomethane and was shown by vapor phase chromatography to have the same retention time on an Apiezon N column at 150° as authentic methyl 3-hydroxydecanoate. No peak corresponding to methyl decanoate could be found.

Taking all of the above physical and chemical evidence into consideration, it is clear that structure II is unique in accommodating all of the properties of serratamolide, which differs from the known depsipeptides[12] such as valinomycin[13] and the enniatins[14] in that it contains β-hydroxy rather than α-hydroxy acid residues.

Serratamolide has been screened for antibiotic activity against a number of test organisms. The following organisms were inhibited at the concentrations indicated.[15]

Organism	Inhibitory concn., μg./ml. 24 hr.	48 hr.
Staphylococcus aureus	50	50
Bacillus subtilis	50	50
Sarcina lutea	6.25	6.25
Mycobacterium tuberculosis	..	25
Mycobacterium avium	..	25
Brucella bronchiseptica	25	>100
Saccharomyces pastorianus	25	>100
Trichophyton rubrum	..	50
Trichophyton interdigitale	..	50

This activity, slight in comparison to the clinically useful antibiotics, is nevertheless of the same order of magnitude as that of the depsipeptide antibiotics isolated from the various strains of *Fusarium*.[16]

Experimental[17]

Isolation of Serratamolide. (i) From Methylene Chloride Extraction of Liquid Cultures.—Sixteen Fernbach style flasks of 2.5-l. capacity were charged with 750 ml. of a solution containing 1.0% glycerol and 0.5% Bacto peptone. The flasks were lightly plugged with cotton, autoclaved for 30 min. at 15–18 p.s.i., and then cooled and inoculated with 50 ml. of a 24-hour culture of *S. marcescens* 9-3-3. The cotton plugs were replaced, and the flasks were aerated by shaking on an Eberbach reciprocating shaker for about 72 hours. The cultures were pooled and extracted twice with one-tenth their total volume of methylene chloride in a large separatory funnel. The methylene chloride extract was washed rapidly with one-tenth of its volume of 1 *N* HCl and then with two one-tenth volume portions of 1 *N* NaOH. It was then washed once with a small quantity of distilled water and dried over anhydrous sodium sulfate. The methylene chloride was removed by distillation under vacuum and the semi-solid residue extracted for 30 min. with 100 ml. of boiling absolute alcohol.

The hot alcohol solution was treated with a little Norite and filtered. On cooling, the filtrate deposited the characteristic yellow-green needles of prodigiosin precursor.[4] The crystals were filtered and the filtrate concentrated to one-half of its original volume and cooled. A small second crop of crystalline precursor was collected. This process was repeated until a volume of 3–4 ml. was reached. No more prodigiosin precursor precipitated, but on standing at −5° for 2 days, large clusters of fine white needles formed. The crystals were dried in vacuum to yield 150 mg. of fine white needles, m.p. 156–157°. A few more mg. of crystals were obtained by concentrating the mother liquor and chilling. The combined crystal crops were recrystallized three times from absolute alcohol. The resulting product was dried at 100° and 0.01 mm. for 6 hours, m.p. 159–160°, $[\alpha]^{25}D$ +4.82° (*c*, 2.5 in ethanol) +7.85° (*c*, 2.3 in chloroform).

Anal. Calcd. for $C_{26}H_{46}O_8N_2$: C, 60.68; H, 9.01; N, 5.44; mol. wt., 514.6; one C–CH_3 per 13 carbon atoms, 5.84 CH_3. Found: C, 60.90; H, 8.86; N, 5.22; mol. wt., 556 (Rast), 543 (ebullioscopic, acetone); CH_3, 6.53; alkoxyl (Zeisel), 0.0.

(11) B. Helferich, *Adv. Carbohydrate Chem.*, **3**, 88 (1948).

(12) See review of depsipeptides by M. M. Shemjakin, *Angew. Chem.*, **72**, 342 (1960).

(13) H. Brockmann and H. Geeren, *Ann.*, **603**, 216 (1957).

(14) P. A. Plattner and U. Nager, *Helv. Chim. Acta*, **31**, 2192 (1948).

(15) Dr. M. Perelman, Eli Lilly and Co., personal communication.

(16) M. S. Lacey, *J. Gen. Microbiol.*, **4**, 122 (1950).

(17) All melting points were taken in open Pyrex capillaries and are uncorrected. A Perkin-Elmer model 21 recording spectrophotometer was used in the determination of the infrared spectra. The Microanalytical Laboratory of the E.T.H., Zurich, Switzerland, performed molecular weight determinations on the di-tetrahydropyranyl and trityl ethers of serratamolide.

The ultraviolet spectrum shows no characteristic absorption bands. End absorption increases gradually from 250 mμ. The infrared spectrum (KBr) shows a strong, sharp band at 3.01 μ, intense bands in C–H region at 3.42 and 3.52 μ, the strongest band in the spectrum at 5.80 μ, a band of almost equal intensity at 6.05 μ and a third strong band at 6.46 μ. In $CHCl_3$ solution the corresponding infrared absorption bands are found at 2.95 (broad), 3.41 and 3.50 μ and at 5.77, 5.96 and 6.53 μ.

(ii) From **Cultures Grown on Nutrient Agar.**—To prepare starting solutions, 500-ml. portions of nutrients containing 0.5% DIFCO Bacto-Peptone and 1% glycerol were placed in 2.5-l. Fernback flasks, loosely stoppered with cotton, autoclaved for 30 min. at 15 p.s.i. and cooled and aerated on a shaker overnight. They were then inoculated with *Serratia* (P–1, 9-3-3 or HY-3) either from lyophilized cultures or from vegetative cultures grown and stored on agar slants containing 2% DIFCO Bacto-Agar in addition to the above nutrients. The starting solutions were incubated on a shaker at room temperature for 24–36 hours before use.

Cultures were allowed to grow for 3 days at room temperature in 2 × 30 × 45 aluminum trays in special wooden cabinets, previously sterilized with 95% alcohol and dried for 1–2 hours with sun-lamps.

The thick paste of bacteria from 30 trays was scraped off the agar and extracted with three 600-ml. portions of acetone. Removal of most of the acetone and dilution with water was followed by extraction with 1500 ml. of ether. The washed and dried ether solution was evaporated, leaving an oily residue. This was washed with warm hexane, yielding a solid which was recrystallized from absolute alcohol. As much as 2 g. of crude serratamolide could be obtained from 30 trays of bacteria, corresponding to approximately 4 square meters of culture.

Serratamic Acid by Mild Hydrolysis of Serratamolide.—Serratamolide (141.5 mg., 0.257 mmole) was dissolved in aqueous sodium hydroxide (5 ml., 1 *N*) at room temperature. Addition of a few drops of acetone accelerated this process by improving the contact between the phases. After all of the serratamolide had dissolved, the solution was cooled and acidified with cold, dilute hydrochloric acid. The crystalline precipitate was collected on a Hirsch funnel, washed with water, and dried in a vacuum desiccator overnight at room temperature; yield 126 mg. (83.5%), m.p. 137–138°, mixed m.p. with authentic serratamic acid 137–138°.[18]

The combined filtrate and washings were extracted with ether (30 ml.), the solvent removed in a stream of nitrogen on a water-bath and the residue crystallized from water. This yielded another 7.0 mg. of serratamic acid, m.p. 137–138°, bringing the total yield to 133 mg. (88%).

Identification of Ethyl 3-Hydroxydecanoate from Acid Hydrolysis of Serratamolide.—Serratamolide (200 mg.) and concentrated hydrochloric acid (2 ml.) were heated on the steam-bath for 2 hours. The resulting mixture was diluted with water (5 ml.) and extracted with ether twice (10 ml. total). The ether layer was washed with water and the ether was removed in a stream of nitrogen on a steam-bath. Aqueous sodium hydroxide (2 ml., 2 *N*) was added to the residue and the mixture was heated on the steam-bath for 30 min. The resulting solution was cooled, acidified with dilute hydrochloric acid and extracted with two portions of ether (10 ml. total). The ether extract was washed with water and with a saturated solution of sodium chloride, and was treated with excess ethereal diazoethane, prepared in solution from N-nitrosoethylurea and a mixture of concentrated potassium hydroxide and ether. The solvent and excess diazoethane were removed in a stream of nitrogen and the residue was injected into a vapor phase chromatograph at 150° equipped with an Apiezon N column. One major peak was obtained which corresponded in retention time to the ethyl ester of 3-hydroxydecanoic acid prepared from octanealdehyde and ethyl bromoacetate by a Reformatsky reaction, according to the procedure used by Harding and Weizmann[19] for the preparation of ethyl 3-hydroxynonanoate. Comparison of the infrared spectra of the two substances after chromatography confirmed their identity.

Acid Hydrolysis of Serratamolide. (i) **Examination of Amino Acid Fragment by Ion Exchange Chromatography.**—Serratamolide (28.09 mg., 54.6 μm., corresponding to 109 μm. of amino acids) was heated with concentrated hydrochloric acid (5 ml.) for 1.5 hours on a steam-bath. The resulting solution was diluted to 25 ml. One ml. of this sample solution was diluted to 5 ml. with a *p*H 2.2 sodium citrate buffer (0.2 *N* in sodium ion) containing caprylic acid, thiodiglycol and a detergent. Two ml. of this buffered solution was assayed in a Beckman/Spinco model 120 amino acid analyzer on a 150-cm. column, using a *p*H 3.25 buffer (0.2 *N* in sodium ion) as eluent; found: 1.59 μmoles of serine (91%). No other amino acids were detected.

(ii) **Examination of Amino Acid Fragment by Paper Chromatography.**—Whatman No. 1 paper was sprayed with a buffer solution made up from 8 ml. of 0.067 *M* KH_2PO_4 and 2 ml. of 0.067 *M* Na_2HPO_4. The paper was dried in an oven at 60°. Serratamolide (66 mg.) was hydrolyzed as above and spotted on the prepared paper along with serine and serine hydrochloride. The spots were dried in a stream of hot air and the paper placed in a descending chromatography jar. The phosphate buffer was shaken with phenol and the aqueous layer was introduced in the bottom of the jar. After allowing the system to equilibrate for 2.5 hours at room temperature, phenol saturated with the phosphate buffer was introduced in the trough in the upper part of the jar, and the chromatogram was allowed to run overnight at room temperature. After removal from the jar it was dried in an oven at 60° for 30 min. and sprayed with 0.25% ninhydrin in acetone and dried in the oven once more in order to develop the spots. All spots were found to have the same R_f value, and no other spots than those due to serine could be found. The same results were obtained when untreated Whatman No. 1 paper and 80% aqueous phenol were used.

Serratamolide Trityl Ether.—Serratamolide (0.20 g.) and trityl chloride (0.30 g.) were dissolved in dry pyridine (5 ml.) and allowed to stand for 3 days at room temperature. At the end of this period the solution was poured into cold water (100 ml.) and extracted with two portions of ether (150 ml. total). The ether extract was washed with water and a saturated solution of sodium chloride, and then dried over anhydrous sodium carbonate overnight. The sodium carbonate was removed by filtration and washed with two small portions of anhydrous ether. Florisil (5 g.) was added to the ether solution and the solvent was removed in a rotary evaporator. The material thus adsorbed on Florisil was introduced on top of a column of Florisil (50 g.), packed in heptane, and the products were eluted with mixtures of heptane–ether and ether–acetone. The fractions eluted by 80:20 and 60:40 mixtures of acetone were combined (0.31 g., 80% theoretical) and recrystallized twice from ethanol for analysis, m.p. 186–187.5°.

Anal. Calcd. for $C_{64}H_{72}O_8N_2$: C, 76.92; H, 7.47; N, 2.80; mol. wt., 999. Found: C, 77.05; H, 7.27; N, 3.06; 3.23, 2.65; mol. wt., 900 (thermoelectric).

Serratamolide Tetrahydropyranyl Ether. (i) **Preparation.**—Serratamolide (0.1 g.) was dissolved in dihydropyran (1.0 ml.), and concentrated hydrochloric acid was added (1 drop). After the vigorous exothermic reaction had subsided, the solution was warmed gently *in vacuo* for 30 min. in order to remove as much of the reagent as possible. The remaining liquid was taken up in methylcyclohexane and chromatographed on alumina (50 g., Fisher 80–200 mesh) using methylcyclohexane–ether and ether–acetone mixtures to elute the bands. The largest fraction was crystallized from acetone–water to give 30.3 mg. (23%) of long, thin needles, m.p. 146–148°.

Anal. Calcd. for $C_{36}H_{62}O_{10}N_2$: C, 63.31; H, 9.15; N, 4.10; mol. wt., 683. Found: C, 63.64; H, 9.19; N, 4.03; mol. wt. (ebullioscopic) 712, (thermoelectric) 610.

(ii) **Acid Hydrolysis to Serratamolide.**—Serratamolide tetrahydropyranyl ether (52.4 mg., 0.77 mmole) was heated in a water-bath with dilute hydrochloric acid (5 ml., *ca.* 1 *N*) and ethanol (2 ml.) for 30 min. The solution was cooled, more cold water was added (2 ml.) and the mixture was stored in the refrigerator overnight. The precipitate was separated by centrifugation, washed with water and dried *in vacuo* for 3 hours at 56°; yield 37.0 mg. (94%) of crude serratamolide, m.p. 152–154° dec. The infrared spectrum was indistinguishable from that of pure serratamolide.

Acetylserratamolide.—Serratamolide (1.0 g.) was dissolved in dry pyridine (10 ml.), and acetic anhydride (4.0

(18) N. J. Cartwright (see ref. 8) reports m.p. 138° dec. for serratamic acid.

(19) V. J. Harding and C. Weizmann, *J. Chem. Soc.*, **97**, 302 (1910).

g.) was added. The solution rapidly turned cloudy, some heat was evolved, and a gelatinous precipitate started to form. After the evolution of heat had ceased, the mixture was warmed on an oil-bath to its boiling point and poured into ice-water (30 ml.). The precipitate was collected by filtration, washed with dilute hydrochloric acid and with distilled water, and air-dried. The crude acetylserratamolide was recrystallized from absolute ethanol to yield 0.9 g. (77%) of thin needles, m.p. 222.5–223° dec.

Anal. Calcd. for $C_{30}H_{50}O_{10}N_2$: C, 60.18; H, 8.42; N, 4.63; mol. wt., 599. Found: C, 60.49; H, 8.49; N, 4.78; mol. wt., 599 (ebullioscopic).

Acetylserratamolide was mixed with aqueous sodium hydroxide (1 *N*, 10 ml.) and ethanol (1 ml.). The mixture was heated on a steam-bath for 30 min., cooled, acidified with dilute hydrochloric acid, and extracted with three portions of ether (30 ml. total). The ether extract was dried over anhydrous sodium sulfate and evaporated. The residue was dried in a vacuum desiccator overnight, washed with a small amount of chloroform and recrystallized from water. The product melted at 137°, and its infrared spectrum was superimposable on that of pure, authentic serratamic acid.

Tosylserratamolide.—Serratamolide (1.44 g.) was dissolved in pyridine (25 ml.), and toluenesulfonyl chloride (1.5 g.) was added to the well-chilled solution. The solution was stored at 0° for 3 hours and then water (5 ml.) was added dropwise with shaking and cooling and the resulting mixture poured into ice-water (200 ml.). The precipitate was removed by filtration while the mixture was still cold and was washed on the filter with very dilute hydrochloric acid and then by large amounts of cold water. The washed precipitate was dried overnight *in vacuo* over Drierite; yield 1.9 g. (83%) of crude tosylserratamolide.

For analysis, the material was recrystallized twice from acetone–hexane. The melting point remained unsharp at about 150° dec.

Anal. Calcd. for $C_{40}H_{58}O_{12}N_2S_2$: C, 58.37; H, 7.10; N, 3.41. Found: C, 58.54; H, 7.43; N, 3.62.

All attempts at further purification led to products showing decomposition as evidenced by marked changes in the infrared spectrum of the material.

Bromoserratamolide.—Serratamolide (1.0 g.) in dry pyridine (15 ml.) was chilled in an ice–salt mixture, and methanesulfonyl chloride (1.0 ml.) was added dropwise and with stirring. The solution was allowed to stand for 3 hours in an ice-bath. Water (1.0 ml.) was added dropwise with cooling and stirring and the mixture was poured into crushed ice and water (250 ml.). The precipitate was collected by filtration and was washed thoroughly with dilute hydrochloric acid, water, and finally a small amount of cold ether. The product, 1.16 g. (87.5%), m.p. 125.5–127° dec., was dried *in vacuo* overnight.

The mesyl derivative was sensitive to heat and solvents and appeared to undergo decomposition during attempts at recrystallization. The crude mesylserratamolide (1.14 g.) was treated with excess lithium bromide (2.0 g.) in dry methyl ethyl ketone (50 ml.) at reflux for 3 hours. The solvent was removed on a steam-bath at the water-pump until a solid started to separate. Cold water (200 ml.) then was added, and the precipitate was collected by filtration. The product was washed thoroughly with distilled water and then with a small portion of cold ether. The filter cake was dried *in vacuo* overnight. The dry product weighed 1.01 g. (93%).

Bromoserratamolide was recrystallized from fresh methyl ethyl ketone. The thin needles decomposed slowly between 170 and 193°.

Anal. Calcd. for $C_{26}H_{44}O_6N_2Br_2$: C, 48.76; H, 6.92; N, 4.37; Br, 24.96. Found: C, 49.13; H, 6.75; N, 4.48; Br, 24.40.

Hydrogenation of Bromoserratamolide and Qualitative Examination of the Hydrolysate.—Bromoserratamolide (27.4 mg.) was stirred in methanol (6 ml.) containing a small amount of aqueous sodium hydroxide (0.2 ml., 1 *N*) and palladium catalyst (*ca.* 20 mg. as the oxide) in an atmosphere of hydrogen. After the uptake of hydrogen had ceased (2.44 ml., 110% theoretical), the catalyst was removed by filtration and washed with another portion of methanol (5 ml.). The combined alcohol solutions were treated with a small amount of hydrochloric acid (2 drops) and evaporated to dryness on a water-bath in a stream of nitrogen. Concentrated hydrochloric acid (6 ml.) was added to the residue, and the mixture was heated on the steam-bath for 2 hours, cooled, and extracted with two portions of ether (10 ml. total).

(i) **Examination of Ether Extract.**—The solvent was removed in a stream of nitrogen on a water-bath, and the residue dissolved in aqueous sodium hydroxide (2 ml., 2 *N*) by warming on the water-bath for 30 min. The solution was cooled, acidified with dilute hydrochloric acid, and extracted with ether (5 ml.). The ether extract was washed with water and with a saturated solution of sodium chloride and treated with an excess of ethereal diazomethane. The solvent and excess reagent were removed in a stream of nitrogen on a water-bath and the residue injected into a vapor phase chromatograph equipped with an Apiezon N column at 150°.

Only one major peak, corresponding in retention time to methyl 3-hydroxydecanoate (prepared by hydrolysis of serratamolide), was found. *No peak corresponding to methyl decanoate was observed.*

(ii) **Examination of Amino Acid Fraction.**—The acidic aqueous layer was spotted on Whatman No. 1 paper along with a standard solution containing alanine (0.1 molar) and serine (0.1 molar). The paper was suspended in a descending chromatography jar, allowed to equilibrate with 80% phenol for 2 hours, and chromatographed overnight (*ca.* 15 hours) with the same solvent. The dried chromatogram was sprayed with ninhydrin in ethanol containing a small amount of pyridine. An intense spot was found for alanine with only a very faint spot corresponding to serine.

Hydrogenation and Hydrolysis of Bromoserratamolide and Assay of Amino Acids Produced.—Bromoserratamolide (20.6 mg., 32.16 μmoles) in ethanol (10 ml.) containing aqueous potassium hydroxide (0.1 ml., 1 *N*) was shaken with hydrogen in the presence of palladium catalyst (21.2 mg. as the oxide). After the uptake of hydrogen had ceased (1.76 ml., approximately theoretical), the catalyst was removed by filtration and washed with an additional portion of ethanol (5 ml.). One drop of concentrated hydrochloric acid was added to the combined filtrates, and the solvent was removed in a stream of nitrogen on the water-bath. Concentrated hydrochloric acid (3.0 ml.) was added to the residue, and the mixture was heated on the water-bath for 1.5 hours. The hydrolysate was diluted to 10 ml. with distilled water.

A *p*H 2.2 sodium citrate buffer, 0.2 *N* in sodium ion, containing caprylic acid, thiodiglycol and a detergent, was used to dilute 2 ml. of the above sample solution (12.87 μ moles of amino acids) to 10 ml. A 2-ml. sample of this buffered solution (2.573 μmoles of amino acids) was assayed as described above for hydrolysis of serratamolide; found, 1.730 μmoles of alanine (67.2%) and 0.068 μmole of serine (2.6%).