acetate was removed under reduced pressure, giving 410 mg (91%) of a pale-yellow, viscous oil, 3-oxo-1,4,6-trienic acid (**3a**). This crude trienoic acid (**3a**) was not purified further, but exhibited the following expected spectral properties: uv λ_{max} (EtOH) 232 nm (ϵ 12 860) and 316 (12 880); ir (film) ν 3500–2500 (broad), 1730, 1650, and 1600 cm^{-1}; NMR δ 1.16 (s, 3, C-10 CH_3), 1.41 (d, J = 7 Hz, 3, C-11 CH_3), 1.95 (s, 3, C-4 CH_3), 3.35 (q, J = 7 Hz, 1, C-11 H), 6.32 (d, J = 10 Hz, 1, C-2 H), 6.80 (d, J = 10 Hz, 1, C-1 H), 6.61 (broad s, 1, C-6 H), and 8.50 (1, COOH).

3a was esterified by treatment with excess etheral diazomethane. The crude product was purified by silica gel column chromatography, affording triene keto ester **3b** as a pale-yellow oil: bp 157–159 °C (7 mm); MS *m/e* (rel intensity) 260 $[M]^+$ (58), 245 $[M - CH_3]^+$ (28), 201 $[M - COOCH_3]^+$ (45), 185 (50), 173 (100); $[\alpha]^{21}D$ +273° (*c* 6.6, $CHCl_3$); uv λ_{max} (EtOH) 235 nm (ϵ 12 050), 310 (12 260); ir (film) ν 1735, 1655, 1615 cm^{-1}; NMR δ 1.16 (s, 3, C-10 CH_3), 1.38 (d, J = 7 Hz, 3, C-11 CH_3), 1.93 (s, 3, C-4 CH_3), 3.31 (q, J = 7 Hz, 1, C-11 H), 3.68 (s, 3, $COOCH_3$), 6.22 (d, J = 10 Hz, 1, C-2 H), 6.73 (d, J = 10 Hz, 1, C-1 H), 6.54 (broad s, $W_{1/2}$ = 4 Hz, 1, C-6 H).

The 2,4-dinitrophenylhydrazone of **3b** was obtained as deep-red plates (**3c**), mp 188–190 °C (from ethanol).

Anal. Calcd for $C_{22}H_{24}O_6N_4$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.76; H, 5.57; N, 12.77.

Treatment of 14-Bromide 6 with Sodium Carbonate. To a solution of 14-bromide **6** (400 mg) in acetone (20 ml) was added 10% sodium carbonate (8 ml). The solution was heated to reflux temperature for 1 h. The reaction mixture was concentrated and diluted with 10% sodium carbonate, and the extracted with ethyl acetate. The extracts were washed with water and dried. Evaporation of the solvent gave 60 mg (12%) of pale-yellow crystals. Recrystallization from ethanol or benzene gave colorless needles: mp 310–312 °C; mass spectrum $[M]^+$ 492.2506 (calcd for $C_{30}H_{36}O_6$, 492.2509), *m/e* (rel intensity) 492 $[M]^+$ (14), 477 $[M - CH_3]^+$ (95), 464 $[M - CO]^+$ (5), 262 (27), 246 $[M/2]^+$ (20), 202 (21), 173 (100); $[\alpha]^{21}D$ −214° (*c* 0.4, $CHCl_3$); uv λ_{max} (EtOH) 243 nm (ϵ 27 700); ir (KBr) ν 1725, 1662, 1630, 1610 cm^{-1}; NMR δ 1.18 (d, J = 7 Hz, 6, C-11,11′ CH_3), 1.31 (s, 6, C-10,10′ CH_3), 4.90 (d, J = 12 Hz, 2, C-14,14′ H_a), 5.00 (d, J = 12 Hz, 2, C-14,14′ H_b), 6.28 (d, J = 12 Hz, 2, C-2,2′ H), 6.78 (d, J = 10 Hz, 2, C-1,1′ H).

Anal. Calcd for $C_{30}H_{36}O_6$: C, 73.14; H, 7.37. Found: C, 73.14; H, 7.50.

Hydrolysis of the Dimer 7. The dimer **7** (40 mg) was dissolved in a solution of potassium hydroxide (500 mg) in methanol (5 ml). The solution was stirred at room temperature for 5 h. The reaction mixture was acidified with hydrochloric acid and then extracted with ethyl acetate. The extracts were washed with water, saturated sodium bicarbonate, and water and dried. Evaporation of the solvent afforded 16 mg of pale-yellow oil. The oily product was a mixture of the hydroxy keto ester **8a** and the methoxy keto ester **9a**, which were identified by means of NMR spectroscopy.

The aqueous solution was acidified and extracted with ethyl acetate. The extracts were washed with water and dried and the solvent evaporated to give 28 mg of pale-yellow oil. The acidic oil, which was a mixture of hydroxycarboxylic acid **8b** and methoxycarboxylic acid **9b**, was not purified further but was treated with ethereal diazomethane. The products and proceeding neutral oil (16 mg) were combined. The viscous oil thus obtained was purified by preparative TLC (solvent hexane–acetone, 1:1).

Band 1 gave 17 mg of the methoxy keto ester **9a** as a pale-yellow oil: MS *m/e* (rel intensity) 292 $[M]^+$ (4), 277 $[M - CH_3]^+$ (31), 260 $[M - CH_3OH]^+$ (16), 201 $[M - (CH_3OH + COOCH_3)]^+$ (19), 173 (100); $[\alpha]^{21}D$ −78° (*c* 0.8, $CHCl_3$); uv λ_{max} (EtOH) 242 nm (ϵ 11 100); ir (film) ν 1735, 1660, 1630 cm^{-1}; NMR δ 1.20 (d, J = 7 Hz, 3, C-11 CH_3), 1.24 (s, 3, C-10 CH_3), 3.32 (s, 3, C-14 OCH_3), 3.70 (s, 3, $COOCH_3$), 4.25 (d, J = 10 Hz, 1, C-14 H_a), 4.29 (d, J = 10 Hz, 1, C-14 H_b), 6.25 (d, J = 10 Hz, 1, C-2 H), 6.72 (d, J = 10 Hz, 1, C-1 H).

Band 2 gave 15 mg of the hydroxy keto ester **8a** as a pale-yellow oil: MS *m/e* (rel intensity) 278 $[M]^+$ (2), 260 $[M - H_2O]^+$ (19), 201 $[M - (H_2O + COOCH_3)]^+$ (19), 173 (100); $[\alpha]^{21}D$ −52° (*c* 3.6, $CHCl_3$); uv λ_{max} (EtOH) 242 nm (ϵ 11 400); ir (film) ν 3440, 1730, 1660, 1625 cm^{-1}; NMR δ 1.21 (d, J = 7 Hz, 3, C-11 CH_3), 1.25 (s, 3, C-10 CH_3), 2.80 (1, OH), 3.70 (s, 3, $COOCH_3$), 4.46 (s, 2, C-14 CH_2), 6.25 (d, J = 10 Hz, 1, C-2 H), 6.80 (d, J = 10 Hz, 1, C-1 H).

Photolysis of the Dimer 7. A solution of 70 mg of **7** in 14 ml of dried dioxane was irradiated in a quartz probe at room temperature with a Rayonet preparative reactor RPR-208 (RUL-3000 Å). The solvent was removed in vacuo and the residue was subjected to preparative TLC (benzene–ethyl acetate, 1:1), which yielded 10 mg of colorless prisms, mp 261–262 °C (**10**). Recrystallization from ethanol gave colorless prisms: mp 263–265 °C, mass spectrum $[M]^+$ 492.2503 (calcd for $C_{30}H_{36}O_6$, 492.2509), *m/e* (rel intensity) 492 $[M]^+$ (1), 246 $[M/2]^+$ (20), 202 (12), 173 (81), 145 (70), 105 (55), 91 (100); uv λ_{max} (EtOH) 234 nm (ϵ 9200); ir (KBr) ν 1730, 1700 cm^{-1}; NMR δ 1.07 (s, 6, C-10,10′ CH_3), 1.08 (d, J = 7 Hz, 6, C-11,11′ CH_3), 4.10 (d, J = 12 Hz, 2, C-14,14′ H_a), 4.85 (d, J = 12 Hz, C-14,14′ H_b), 5.95 (d, J = 5 Hz, 2, C-2,2′ H), 7.35 (d, J = 5 Hz, 2, C-1,1′ H).

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Registry No.—**2,** 17974-84-4; **3a,** 57901-33-4; **3b,** 57901-34-5; **3c,** 57901-35-6; **4,** 57901-36-7; **5,** 57901-37-8; **6,** 57901-38-9; **7,** 57901-39-0; **8a,** 57901-40-3; **9a,** 57901-41-4; **10,** 57901-42-5; (−)-6-epi-α-santonin, 1618-78-6; *N*-bromosuccinimide, 128-08-5.

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Electrochemical Oxidation of Halomicin B to Rifamycin S

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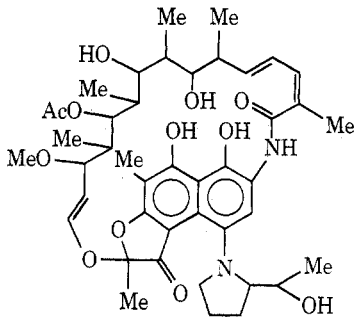
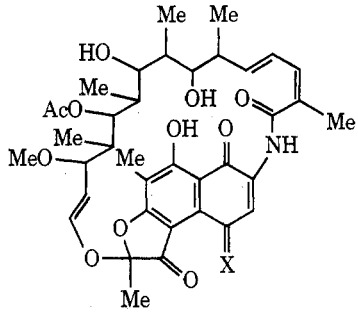
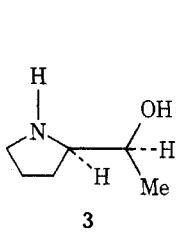
In an earlier communication[1] we have disclosed the structure of halomicin B (**1**). Elucidation of the structure of halomicin groups of antibiotics involves their conversion to a rifamycin S derivative and a substituted pyrrolidine base. Thus, the structural elucidation of halomicin B (**1**) involved its conversion into rifamycin S (**2**)[2] and a basic component (**3**).[1] In connection with the above-mentioned work, we have studied electrochemical oxidation of this group of antibiotics. It was conceived that halomicin B on electrochemical oxidation will lose two electrons easily and will be converted into a cationic species (**4**) which upon work-up will hydrolyze spontaneously into rifamycin S (**2**) and compound **3**. A similar cation (**4**) could also be produced from **1** in two steps: losing the first electron will yield a cation radical which could be trapped by a nucleophile (inter- or intramolecularly) into a radical followed by the loss of a second electron to the cation (**4**). The controlled potential[3] electrochemical oxidation of halomicin B was carried out in



1



2, X = O
6, X = -OCH$_2$C(=O)-O-



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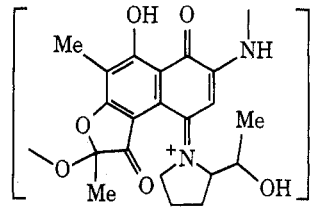

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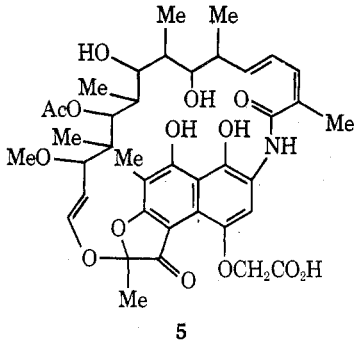
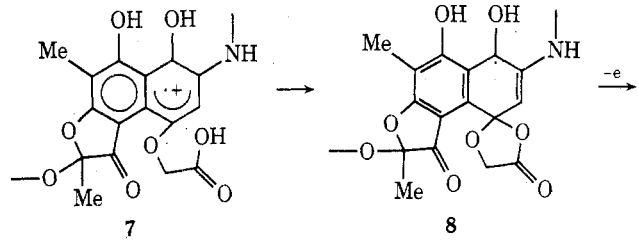


5

pH 4 buffer solution. The oxidation proceeds less efficiently at pH 6 and 7. After the oxidation was complete the reaction mixture was concentrated to a small volume. Extraction with chloroform followed by preparative TLC yielded pure rifamycin S (yield ~90%). The aqueous layer was basified and then extracted with ether to yield **3** (detected by TLC). Rifamycin B (**5**) on similar electrochemical oxidation was converted into rifamycin O (**6**) in a quantitative yield. This conversion could also proceed by the loss of two electrons directly or stepwise through the intermediacy of a radical cation (**7**) → radical (**8**) followed by its oxidation to the cation **9** which loses a proton to yield compound **6**.

During our work on the structural elucidation of halomicin B, we observed that preparative TLC (Analtech, silica gel GF) of rifamycin S (it is yellow in color as reported in the literature[2]) yielded a deep red colored crystalline compound which has been shown to have structure **10** and will be referred to in this communication as rifamycin S (red). Rifamycin S (red), $C_{37}H_{45}O_{12}N$: $[\theta]_{435}$ +25 000; λ_{max} 226



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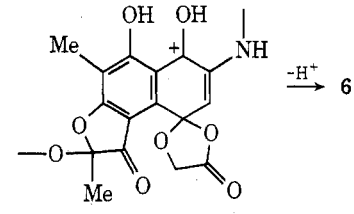

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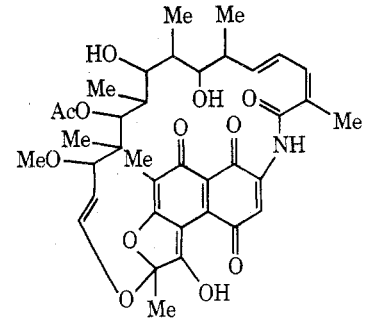

10

nm (ϵ 29 800), 265 (20 400), 312 (23 600), 532 nm (423); ν_{max} 3500, 3333, 1715, 1627, 1613 cm^{-1}. Rifamycin S (red) in THF solution when treated with a catalytic amount of acid was converted to rifamycin S (yellow). This interconversion suggested that the two compounds could be tautomeric. The NMR spectrum of rifamycin S (yellow) showed signals at δ 2.33 (aromatic methyl), H_3 at δ 7.8, and the hydrogen-bonded phenolic hydroxyl group at δ 12.5 whereas in rifamycin S (red) the aromatic methyl group appeared at δ 2.31 and H_3 at δ 7.64 and the signal at δ 12.5 was absent.[4] These results are consistent with the assigned structures **2** and **10** for rifamycin S yellow and red, respectively. Both these compounds have similar biological activities.

Experimental Section

Electrochemical Oxidation of Halomicin B (1) to Rifamycin S (2). A solution of halomicin B (100 mg) in a mixture of methanol and aqueous acetate buffer pH 4 (1:1 v/v) was deaerated for 1 h prior to oxidation using argon saturated with the above solvent mixture. The electrolysis was run at +0.3 V (vs. SCE) at 25 °C until there was no change in the integrated electrolysis current. The reaction mixture was concentrated to a small volume and then extracted with chloroform to yield rifamycin S (90 mg), shown to be identical with an authentic sample (mixture melting point, $[\alpha]_D$, uv, ir, NMR, and MS). The aqueous layer on basification and extraction with ether yielded **3** (detected by TLC and direct comparison with an authentic sample).

Electrochemical Oxidation of Rifamycin B (5) to Rifamycin O (6). It was carried out exactly as above and the yield of rifamycin O from rifamycin B was quantitative. The identity of rifamycin O was established by direct comparison with an authentic sample.

Registry No.—1, 54356-09-1; 2, 13553-79-2; 5, 13929-35-6; 6, 14487-05-9; 10, 57821-04-2.

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(3) Controlled-potential electrolytic oxidations were carried out using a Princeton Applied Research (Princeton, N.J.) Model 170 electrochemistry system with an X-Y recorder display of the integrated electrolysis cur-

rent and a platinum working electrode cell [L. P. Rigdon and J. E. Harrar, *Anal. Chem.*, **46**, 696 (1974)] with aqueous pH 4 acetate buffer–methanol (1:1 v/v) in the salt bridge and the cathode compartment.

(4) NMR also shows the presence of exchangeable amide proton, thus ruling out other tautomeric structures.

Selective Reduction of the Amide Carbonyl Group in Dipeptides by Borane[1]

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The facile reduction of amides by borane[2,3] led us to attempt the reduction of the peptide carbonyl group in *N*-alkoxycarbonyl dipeptide esters **1** in order to obtain the corresponding diamino esters **2.** The products, after hydrolysis of the ester and suitable protection of the newly generated amino group, can be regarded as derivatives of diamino acids **4** and could be incorporated into synthetic peptides. This would amount to selective replacement of a peptide bond by an aminoethylene unit (–CONH → –CH_2NH–) and would be useful in structure–activity studies in peptide hormones. The amino group also offers a point of attachment of a reactive group for affinity labeling of enzymes and receptors.

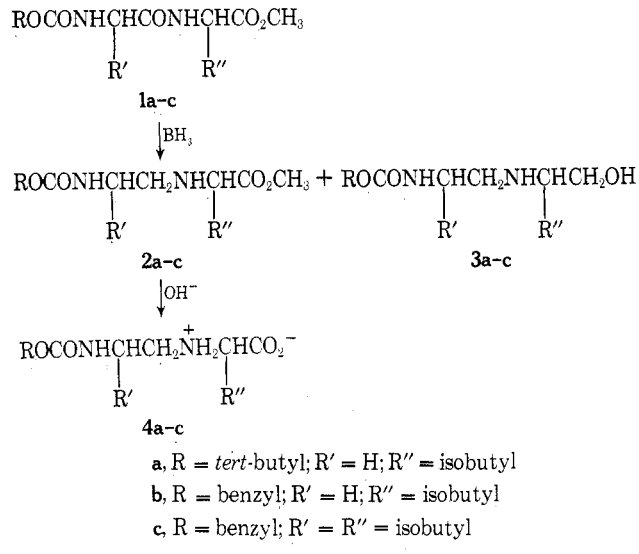
Since the relative ease of reduction of isolated carbonyl groups by BH_3 is $-CO_2H > -CONR_2 > CONHR > -CONH_2 > -CO_2R >$ ROCONHR,[2-4] it was a priori possible to reduce selectively the amide bond in **1.** However, an ester group located α to an amide is more easily reduced to the corresponding alcohol than is an isolated ester. Thus when benzoyl glycine ethyl ester was treated with borane, the amino ester and amino alcohol were produced in yields of 11 and 85%, respectively.[3]

We have found that concurrent reduction of the ester in dipeptides is minimized by carrying out the reaction at −20 °C with 2 mol of BH_3 for 4–5 h. Under these conditions, a considerable amount of starting material remains unreacted, but it is easily separated from the basic products and does not diminish greatly the synthetic usefulness of the procedure. Thus, reduction of 3.0 g of Boc-Gly-Leu-OMe[5] under the above conditions gave 1.24 g of starting material and 0.64 g of pure **2a,** after separation from other products by chromatography on silica gel. Alkaline hydrolysis of **2a** gave the amino acid **4a** in 73% yield. In general, more vigorous reaction conditions led to more complex mixtures (TLC) from which only the amino alcohols **3** could be isolated.

We have found that the severe acid treatment used to hydrolyze the amine–borane complexes formed after reduction of simple amides[2] can be replaced by treatment with 0.5 N HCl in methanol at room temperature overnight, which is compatible with the benzyloxycarbonyl group. In fact, reductions of the acid-sensitive Boc dipeptide esters were quenched by treatment with aqueous methanol overnight.

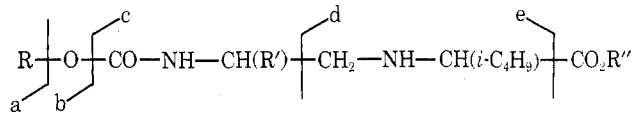
Although we expect the reduction to proceed with complete retention of asymmetry at the chiral centers, we have not proved this point. Perhaps the best evidence for retention is that the products from several reductions of the same material have the same optical rotations and have sharp melting points.

Scheme I



a, R = *tert*-butyl; R′ = H; R″ = isobutyl
b, R = benzyl; R′ = H; R″ = isobutyl
c, R = benzyl; R′ = R″ = isobutyl

The mass spectra of **2a–c** exhibit characteristic fragmentations which can be generalized as



Other ions may appear at M^+, M + 1, b + H, and e − (b + H), or may be due to losses of C_3H_6 (42 amu) or C_4H_8 (56 amu) via McLafferty rearrangements. The most interesting ion in these spectra is ion d. It was previously reported[6] that for polyamino alcohols formed by exhaustive reduction of peptides by $LiAlH_4$, the amine fragment formed by carbon–carbon cleavage was a significant ion in the mass spectra. However, in the present case the charge remains only on the carboxyl fragment.

Experimental Section

Melting points are uncorrected. TLC was carried out on silica gel plates (E. Merck) in BAW (*n*-BuOH–AcOH–H_2O, 4:1:1), TCW (tetrahydrofuran–cyclohexane–H_2O, 93:7:5), EAE (EtOAc–EtOH–AcOH, 9:1:1), and CMA ($CHCl_3$–MeOH–AcOH, 90:30:5); spots were located with ninhydrin and by chlorination followed by starch–iodide spray. Column chromatography was carried out on silica gel 60 (E. Merck) in a column 18 × 1 in. ^1H NMR spectra were taken in a Varian T-60 spectrometer. Mass spectra were determined on an LKB-9000-S at an ionization potential of 70 eV. Tetrahydrofuran was dried by distillation from CaH_2. Borane was obtained from the Aldrich Chemical Co. as a 1 M solution in THF containing 5 mol % $NaBH_4$ stabilizer. All evaporations were done at ≤40 °C. Microanalyses were by Midwest Microlabs, Indianapolis, Ind.

***N*-[2-[(*tert*-Butyloxycarbonyl)amino]ethyl]-L-leucine Methyl Ester Hydrochloride (2a).** A solution of 3.0 g (10 mmol) of Boc-Gly-Leu-OMe in 20 ml of THF was cooled to −20 °C in a flask flushed with N_2 and fitted with a rubber septum. A solution of 20 ml of 1 M BH_3 in THF (Aldrich Co.) was added with a syringe and the reaction was allowed to stir at −20 °C for 4 h under N_2. Residual BH_3 was quenched by cautious addition of 10 ml of MeOH at −20 °C (caution, $H_2\uparrow$) and the mixture was stirred overnight at room temperature; the solution was evaporated under vacuum and treated with MeOH (3 × 50 ml) with evaporation to dryness after each addition, to remove boric acid as trimethyl borate. The residue was suspended in water, the pH was adjusted to 3.0 with HCl, and unreacted starting material (1.2 g) was removed by extraction with ether. The aqueous solution (mixture of **2a** and **3a**) was evaporated to dryness and chromatographed on a column of silica gel. **2a** was eluted with 5% MeOH in $CHCl_3$, followed closely by **3a.** Crystallization from MeOH–Et_2O gave 0.97 g of **2a,** mp 162–164 °C, in 30% yield (51% based on consumed **1a**): $[\alpha]^{25}D$ +9.1° (*c* 1.0, MeOH); TLC (EAE) R_f 0.42; ^1H NMR ($CDCl_3$, Me_4Si) δ 3.83 (s, 3 H, $COOCH_3$), 1.45 [s, 9 H, $-NHCOOC(CH_3)_3$],