Experimental Section[6]

5-Acetoxyvaleraldehyde.[7]—5-Bromo-*n*-pentyl acetate[8] (83.6 g, 0.40 mol), pyridine oxide (76.0 g, 0.80 mol), and sodium bicarbonate (67.2 g, 0.80 mol) were heated at reflux with vigorous stirring in toluene (500 ml) under nitrogen for 4 hr. The mixture was cooled and poured into water (2 l.). The organic layer was separated and the water layer was extracted with toluene. The combined toluene solution was distilled on a spinning-band column to give 28.6 g, (50%): bp 98° (12 mm); n^{28}D 1.4270; dinitrophenylhydrazone, mp 98–99° (lit.[9] mp 100°); nmr ($CDCl_3$) τ 0.23 (t, 1, J = 1.5 Hz), 5.93 (m, 2), 7.49 (m, 2), 7.96 (s, 3), and 8.31 (m, 4).

The same procedure was followed with 5-iodo-*n*-pentyl acetate to give the aldehyde in 40% yield.

Wittig Reaction.—An ether solution of phenyllithium[10] (0.040 mol, 1 *N*) was added to dry tetrahydrofuran (100 ml) under nitrogen. Dry tetramethylene-1,4-bistriphenylphosphonium bromide[11] (14.68 g, 0.020 mol) was added over 5 min at 0–5° and stirred for 5 min more to give a dark yellow color.[12] 4-Heptanone (2.28 g, 0.020 mol) was added over 3 min and stirred for 7 min more at 10°. 5-Acetoxyvaleraldehyde (2.88 g, 0.020 mol) was added over 2 min and stirred for 15 min at 10–18°. This was poured into water (500 ml) and extracted with ether. The extract was evaporated, and hexane (50 ml) was added to the residue to precipitate the triphenylphosphine oxide. The hexane solution was filtered, evaporated, and chromatographed on a column of Woelm acid-washed alumina. Elution with hexane gave 4,9-di-*n*-propyl-4,8-dodecadiene and biphenyl. The diene was purified by vpc, giving 206 mg (4.1%): n^{24}D 1.4618; nmr ($CDCl_3$) τ 4.87 (t, 2), 8.03 (m, 12), 8.61 (m, 8), and 9.13 (t, 12).

Anal. Calcd for $C_{18}H_{34}$: C, 86.3; H, 13.7. Found: C, 86.5; H, 13.5.

Elution with 20% chloroform in hexane gave a mixture of *cis*- and *trans*-10-*n*-propyl-5,9-tridecadienyl acetate, which was distilled [120° (0.05 mm)] giving 445 mg (8%).

Elution with 40% chloroform in hexane gave 5,9-tetradecadiene-1,14-diol diacetate (280 mg, 4.5%). A sample was purified by vpc: n^{24}D 1.4621; ir (neat) 1745 (ester C=O) and 966 cm^{-1} (*trans* C=C); nmr ($CDCl_3$) τ 4.63 (t, 4), 5.94 (t, 4), 7.9 (m, 8), 7.97 (s, 6), and 8.47 (m, 8). Thin layer chromatography on $AgNO_3$–silica gel[13] showed that all three geometrical isomers were present.

Anal. Calcd for $C_{18}H_{30}O_4$: C, 69.64; H, 9.74. Found: C, 69.71; H, 9.84.

Separation of *cis* and *trans* Isomers.—The 10-*n*-propyl-5,9-tridecadienyl acetate prepared above was chromatographed on a column of $AgNO_3$–silica gel[14] and eluted with 3% ether in hexane. The first fractions contained the pure *trans* isomer (110 mg, 2.0%): n^{24}D 1.4599 (lit.[5] n^{24}D 1.4610); ir (neat) 1745 and 966 cm^{-1}. The later fractions contained the pure *cis* isomer (300 mg, 5.3%): n^{24}D 1.4606; ir (neat) 1742 cm^{-1}; nmr ($CDCl_3$) τ 4.62 (t, 2), 4.88 (t, 1), 5.93 (t, 2), 7.97 (s, 3), 8.0 (m, 10), 8.6 (m, 8), and 9.12 (t, 6).

Anal. Calcd for $C_{18}H_{32}O_2$: C, 77.09; H, 11.50. Found: C, 77.27; H, 11.52. The isomer purity of the fractions was determined by thin layer chromatography on $AgNO_3$–silica gel, using sulfuric acid detection.

Conversion of *cis* to *trans* Isomer.[15]—10-*n*-Propyl-*cis*-5,9-tridecadienyl acetate (200 mg) and powdered selenium (10 mg) were heated in an evacuated sealed tube at 220–225° for 1 hr. Distillation at 0.1 mm and 120° afforded 190 mg of 10-*n*-propyl-*trans*-5,9-tridecadienyl acetate containing about 5% of the *cis* isomer by infrared analysis. The small amount of *cis* isomer was removed by chromatography on $AgNO_3$–silica gel as described above to give 170 mg (85%) of pure *trans* isomer.

Registry No.—1, 19889-82-8; 2, 10297-61-7; 3, 22142-01-4; 4, 22142-00-3.

Acknowledgment.—The author wishes to thank M. Jacobson for the biological evaluation, S. W. Bany for technical assistance, and J. J. McBrady for infrared and nmr interpretation.

(6) The nmr spectra were obtained on a Varian Associates A-60 spectrometer, and the vpc analyses and purifications were carried out on a 6 ft × 0.25 in. column containing 10% SE-30 on Chromosorb P or 5% Carbowax 20M on Anakrom ABS. Elemental analyses were performed by P. B. Olson of 3M Co., or Galbraith Laboratories, Inc.

(7) This method is similar to that used to prepare 4-acetoxybutanal from 4-bromobutanol acetate: L. D. Bergel'son, V. A. Vaver, A. A. Bezzubov, and M. M. Shemyakin, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1453 (1964); *Chem. Abstr.*, **64**, 14086*d* (1966).

(8) J. F. Oughton, U. S. Patent 3,112,330 (1963); *Chem. Abstr.*, **60**, P4011*h* (1964).

(9) E. Kobayashi and A. Yuzawa, Japanese Patent 3963 (1961); *Chem. Abstr.*, **59**, P2651*b* (1963).

(10) R. G. Jones, *Org. Reactions*, **6**, 353 (1951).

(11) L. Horner, H. Hoffman, W. Klink, H. Ertel, and U. G. Toscano, *Ber.*, **95**, 581 (1962).

(12) D. W. Dicker and M. C. Whiting, *J. Chem. Soc.*, 1944 (1958).

(13) L. J. Morris, *Chem. Ind.* (London), 1238 (1962).

(14) Prepared by drying a mixture of 20 g of silica gel and 60 ml of 10% aqueous $AgNO_3$ in a rotary evaporator and then heating in an oven at 105° for 1 hr.

(15) This procedure was adapted from that used to isomerize oleic acid: D. Swern and J. T. Scanlan, *Biochem. Prep.*, **3**, 118 (1963).

Improved Synthesis of Streptozotocin

EDWARD J. HESSLER AND HEINZ K. JAHNKE

The Upjohn Company, Kalamazoo, Michigan

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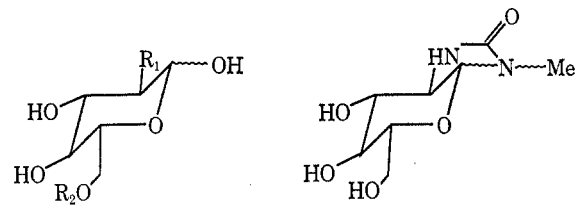
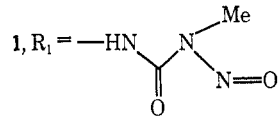
Streptozotocin[1] is a broad-spectrum antibiotic and an antitumor agent produced by *Streptomyces achromogenes*. Streptozotocin (**1**)[2] is an N-nitrosated methylurea derivative of D-glucosamine (**2**). We reinvestigated the synthesis of **1** because of its antibiotic properties, its structural simplicity, and its high cost of production by fermentation.

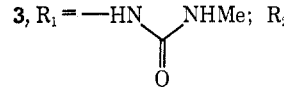


5

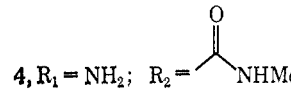


1, R_1 = —HN(Me)N=O; R_2 = H

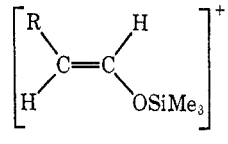
2, R_1 = —NH_2; R_2 = H



3, R_1 = —HN(C=O)NHMe; R_2 = H



4, R_1 = NH_2; R_2 = C(=O)NHMe



6, R = NH_2

7, R = NHCONHMe

(1) (a) J. J. Vavra, C. DeBoer, A. Dietz, L. J. Hanka, and W. T. Sokolski, *Antibiot. Ann.*, **1959–1960**, 230 (1960); (b) W. T. Sokolski, J. J. Vavra, and L. J. Hanka, *ibid.*, **1959–1960**, 241 (1960); (c) C. Lewis and A. R. Barbiers, *ibid.*, **1959–1960**, 247 (1960); (d) R. R. Herr, T. E. Eble, M. E. Bergy, and H. K. Jahnke, *ibid.*, **1959–1960**, 236 (1960); (e) M. E. Bergy, C. De Boer, A. Dietz, T. E. Eble, R. R. Herr, and L. E. Johnson, U. S. Patent 3,027,300 (March 27, 1962).

(2) R. R. Herr, H. K. Jahnke, and A. D. Argoudelis, *J. Amer. Chem. Soc.*, **89**, 4808 (1967).

The earlier reported synthesis[2] of **1** had used acetyl groups for protection of the hydroxyl groups during formation of the N-methylurea and during formation of the N-nitroso urea, but their removal was accompanied by severe degradation. To avoid this, we investigated the direct reaction of **2** and N-methyl isocyanate (MIC).

It was found that if MIC was added to a cold aqueous solution of **2** (free base), a high yield of **3** resulted. Infrared and mass spectra showed that the expected by-product **4** was not present and that **5** was present in only trace amounts. The infrared spectrum of the product revealed a strong band at 1645 cm^{-1}; we assigned this to the open-chain urea.[3] Supporting evidence for this was a strong amide II band[4a] at 1580 cm^{-1}. The infrared spectrum of authentic bicyclic urea **5**[5] has a carbonyl peak at 1660 cm^{-1} and no amide II band[4a] in the region 1500–1600 cm^{-1}. The product could not be a urethan compound such as **4** because the carbonyl absorption of a urethan occurs at *ca.* 1710–1740 cm^{-1}.[4b]

Vapor phase chromatography (vpc) of the trimethylsilyl (TMS) derivative[6,7] of **3** showed two major peaks. Vpc–mass spectrometry[8a] of the TMS derivative gave identical spectra for each major peak, confirming that they were anomeric. A weak *m/e* 524 peak was assigned to the molecular ion; a *m/e* 509 peak [(M − 15)$^+$], a *m/e* 450 peak [(M − 74)$^+$], and an intense *m/e* 188 peak were present. The mass spectrum of the TMS derivative of **2**[6,7] showed an intense *m/e* 131 peak which is due to the C_1–C_2 fragment **6**. We assigned the *m/e* 188 peak of the TMS derivative of **3** to **7**. A high-resolution mass determination[8b] of the *m/e* 450 peak gave a mass of 450.2144 corresponding to $C_{15}H_{42}O_5Si_4$ (calculated, 450.2105). Presumably this is the sugar fragment after a McLafferty rearrangement type cleavage of the urea moiety. These data confirm the presence of the urea moiety in **3**. The nmr spectrum of the trimethylsilyl derivative of **3** gave an N-methyl peak at τ 7.25 and integrated for four trimethylsilyl groups.

When the temperature of the reaction was kept between −2 and 2°, 90–95% of the desired urea **3** resulted. However, if the temperature went above 12° during the addition of MIC to the reaction, the yield of **3** was reduced to *ca.* 40% and unidentified products of higher molecular weight (as shown by mass spectrometry) were formed.

The crude product **3** was treated with nitrous acid generated from commercial nitrogen trioxide[9] to give **1** in 77–80% overall yield from **2**. The synthetic and natural products were shown to be identical by uv and ir spectroscopy, bioassay,[10] and tlc. In addition, synthetic **1** was shown to be diabetogenic in male rats,[11] and anti-leukemic in mice.[12] These physiological properties of synthetic **1** correspond in every way to **1** obtained by fermentation.

(3) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley & Sons, Inc., New York, N. Y., 1958, p 223.

(4) (a) Reference 3, pp 219–220; (b) p 222.

(5) Kindly provided by A. D. Argoudelis, who prepared it from natural streptozotocin (see ref 2).

(6) The conditions referred to are: 10-mg sample, 1.0 ml of dry pyridine, 0.2 ml of hexamethyldisilazane, and 0.1 ml of trimethylsilyl chloride shaken for 1 hr at room temperature; *cf.* C. C. Sweeley, R. Beltley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, **85**, 2497 (1963).

(7) The TMS derivative was isolated by the method reported by B. T. Golding, R. W. Richards, and M. Berber, *Tetrahedron Lett.*, 2615 (1964).

(8) (a) An LKB-9000 mass spectrometer was used under the direction of Dr. P. Bowman, The Upjohn Co.; (b) a CEC-21-110 mass spectrometer was used under the direction of R. Wnuk, The Upjohn Co.

(9) Nitrogen trioxide was purchased from the Matheson Co.

(10) Bioassay was performed as a paper disc agar diffusion with the test organism *Proteus vulgaris*.

Experimental Section

D-Glucosamine free base (**2**) was prepared from the hydrochloride salt as described by Breuer.[13]

D-Glucosamine N-Methylurea (**3**).—A solution of **2** (179.0 g, 1.0 mol) in water (800 ml) was cooled to −2°. Freshly distilled MIC (65.0 ml, 63.0 g, 1.10 mol) was added slowly over a period of 30 min, so that the temperature of the stirred solution did not exceed 2°. After the solution was stirred for 1 hr at 0°, an aliquot (50 μl) was removed, and converted to the TMS derivative[6] for vpc. This showed the presence of the anomeric urea mixture **3**. The urea could be isolated by lyophilization. The vpc conditions are: 3% OV-1 on gas Chromosorb Q, 100/120 mesh, 6 ft × 0.25 in. column, 190°; on a Hewlett–Packard Model 402, with a flame ionization detector.

Streptozotocin (**1**).—The urea **3** was converted to **1** without isolation. Liquid nitrogen trioxide (27 ml, 39.0 g, 0.51 mol) was added to the urea solution over a period of 5 min with stirring at 0°. After the solution was stirred further for 15 min, an aliquot was removed for tlc (50 μl solution diluted to 1 ml with 1:1 methanol–water). The diluted solution was spotted on a microslide and developed in 1:3:1 ethanol–ethyl acetate–cyclohexane. Visualization of the components was accomplished in an iodine chamber. A low-R_f component (R_f *ca.* 0.1) corresponding to **3** and a high-R_f component (R_f *ca.* 0.6) corresponding to **1** were present. More liquid nitrogen trioxide (*ca.* 42 g) was added until the tlc assay indicated that **3** had completely reacted. Cold 1-butanol (4 l.) was added, and at a bath temperature of 35° the mixture was concentrated under reduced pressure to remove the water. During the concentration **1** crystallized. The concentrate (*ca.* 2 l.) was stored at −10° for 3 hr. Compound **1** was collected by filtration; it was washed with 1:1 butanol–ether and ether, and dried to yield 213 g (80% from **2**) of pale yellow crystals, mp 115–115.5° dec. Elemental analysis was acceptable.

Registry No.—**1**, 18883-66-4.

Acknowledgment.—Valuable discussions with Professor C. Sweeley, Dr. M. Grostic, and Dr. P. Bowman regarding mass spectral data were appreciated. Technical assistance was provided by J. E. Wiersma.

(11) W. E. Dulin, private communication.

(12) B. K. Bhuyan, private communication.

(13) R. Breuer, *Chem. Ber.*, **31**, 2193 (1898).

Rotenoids. XXII. Total Synthesis of Isomillettone[1]

MASATERU MIYANO

Chemical Research Division, G. D. Searle & Company, Chicago, Illinois 60680

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A number of 3,4-methylenedioxyphenyl compounds are known[2] to be excellent synergists for pyrethroids, the insecticidal principles of pyrethrum flowers. Enhanced mitocidal activity of a 3,4-methylenedioxyphenyl compound when applied in conjunction with

(1) Part XXI: M. Miyano, *J. Amer. Chem. Soc.*, **87**, 3962 (1965).

(2) For example, see (a) M. Ohno, M. Hamada, and H. Takahara, *Bull. Inst. Chem. Res. Kyoto Univ.*, **38**, 34 (1960); *Chem. Abstr.*, **55**, 22275 (1961); (b) M. Beroza, *Anal. Chem.*, **28**, 1550 (1956); (c) R. Nash, *Ann. Appl. Biol.*, **41**, 652 (1954); *Chem. Abstr.*, **49**, 4221 (1955); (d) O. F. Hedenburg, U. S. Patent 2,485,600 (1949); *Chem. Abstr.*, **44**, 5522 (1950).