Synthesis of Isobufalin Methyl Ester†

T. R. KASTURI, G. R. PETTIT,* and K. A. JAEGGI

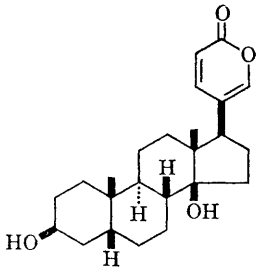
(*Departments of Chemistry, Arizona State University, Tempe, Arizona* 85281, *and University of Maine, Orono, Maine* 04473)

FROM antiquity external application of Ch'an Su, the dried venom of a common toad, appears to have been used by the Chinese for various medical purposes. The venom is now known to be a complex mixture of sterols, bufadienolides, proteins, and a variety of nitrogen heterocyclic compounds.[2] Bufalin (I),[3] one of several bufadienolides isolated from Ch'an Su, can be converted into an isomeric methyl ester derivative‡ [(IIa), m.p. 210—213°, rotatory dispersion: 588 (−100°), 500 (−130°), 450 (−210°), 400 (−350°), 350 (−1000°) mμ, λ_{max} 293 mμ (ε 27,520), i.r. (Nujol): 3530 (OH), 1698 (CO_2Me), 1598 (C=C–O–), 1160 (C–O) cm.$^{-1}$, n.m.r.: 1·0 (angular CH_3), 3·73 (CO_2Me), 4·13 (3α-H), 5·63 (23-H; *J* 15 c./sec.) 6·58 (21-H), 7·23 (22-H; *J* 15 c./sec.) δ] upon treatment[4] with sodium methoxide in dry methanol. We now report chemical conversion of digitoxigenin[5] into 3β-acetoxyisobufalin methyl ester (IIb). Construction of ester (IIb) confirms the structure and D-ring stereochemistry assigned to bufalin, and represents the first total synthesis to approach so closely a naturally occurring bufadienolide.

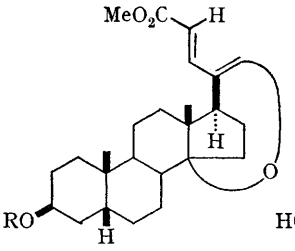
Digitoxigenin [(III), total synthesis reported in

† See ref. 1 for previous paper in this series. This investigation was supported by Public Health Service Grants from the National Cancer Institute.

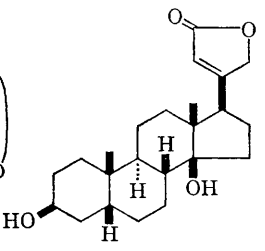
‡ All new compounds gave satisfactory elemental analyses. Optical rotations were measured in $CHCl_3$ and u.v. spectra in cyclohexane solution. N.m.r. spectra were obtained with a Varian A-60 spectrometer ($CDCl_3$ as solvent and tetramethylsilane as internal standard). O.r.d. curves were obtained ($CHCl_3$ solution) using a Jasco Model O.R.D./U.V-5 recorder. Acetylations were carried out with acetic anhydride–pyridine (1 : 3) at room temperature for 20 hr.



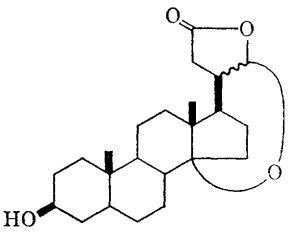
(I)



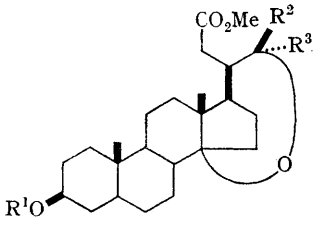
(II) a; R=H
b; R=Ac



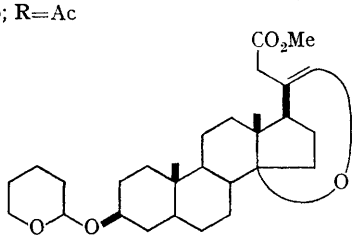
(III)



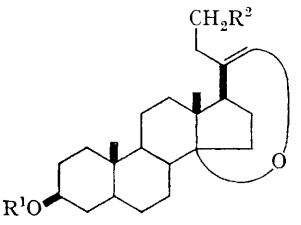
(IV)



(V) a; R^1=Ac, R^2=H, R^3=OMe
b; R^1=Ac, R^2=OMe, R^3=H
c; R^1=2-tetrahydropyranyl, R^2=OMe, R^3=H



(VI)



(VII) a; R^1=2-tetrahydropyranyl R^2=CN
b; R^1=Ac, R^2=CO_2Me

ref. 5] was transformed *via* isodigitoxigenin (IV) into the epimeric acetals (V) as previously reported.[6] Further support for the stereochemistry assigned to isomers (Va) (m.p. 194—196°) and (Vb) (m.p. 142—144°) was provided in the present investigation. Saponification of (Va) (2·0 g.) with 5% methanolic potassium hydroxide for 5 hr., followed by acidification, esterification (ethereal diazomethane), and preparation of the C-3 pyranyl ether (1·2 ml. dihydropyran, 50 mg. toluene-*p*-sulphonic acid, 20 ml. dry benzene at room temperature 1 hr.) afforded exclusively vinyl ether (VI) (1·3 g.), m.p. 125—126°, $[\alpha]_D^{20}$ — 28·8°, n.m.r.: 0·97 (18-CH_3), 1·05 (19-CH_3), 2·88 (22-H), 3·65 (CO_2CH_3), 5·97 (21-H) δ. Under the same reaction conditions, epimer (Vb) gave derivative (Vc), m.p. 128—130°, $[\alpha]_D^{20}$ + 31°, n.m.r.: 0·97 (18-CH_3), 1·08 (19-CH_3), 3·44 (21-OCH_3), 3·66 (CO_2CH_3), 4·21 (21-H, *J* 7·9 c./sec.) δ. Ease of elimination of the methoxy-group in the case of (Va) indicates a *trans*-diaxial relationship to the 20-H. Lithium aluminium hydride reduction of ester (VI) (in refluxing tetrahydrofuran) gave the corresponding alcohol. The alcohol (0·4 g.) was converted into the mesylate (0·28 g.) by treatment with methanesulphonyl chloride–pyridine at 0° for 20 hr. The crude mesylate (0·28 g.) was stirred with an excess (3 mole) of sodium cyanide in anhydrous dimethylformamide (5 ml.) at 40° for 20 hr.[7] to give nitrile (VIIa) (0·16 g.) m.p. 135—137°, $[\alpha]_D^{20}$ — 45·8° (0·3%), i.r.: (Nujol) 2250 (C≡N), 1604 (C=C) cm.$^{-1}$, n.m.r.: 0·97 (18-CH_3), 1·03 (19-CH_3), 3·33—4·0 (3H), and 4·65 [one 3α-H and three pyranyl ether protons], 5·98 (21-H) δ, mass spec.: 366 (M — C_5H_8O) and 351 (M — $C_5H_{10}O_2$). Stirring the nitrile (VIIa) (0·3 g.) with toluene-*p*-sulphonic acid (0·04 g.) in methanol (20 ml.) for 20 hr. cleaved (^{1}H n.m.r. evidence) the pyranyl ether. The crude hydroxy-nitrile (0·28 g.) was heated at reflux (under nitrogen) with potassium hydroxide (0·75 g.) in ethylene glycol (10 ml.) for 6 hr., acidified, and the resulting acid esterified (ethereal diazomethane) and acetylated to yield (VIIb) (0·1 g.), oil, i.r. (neat): 1735 (CO_2Me) and (COMe), 1603 (C=C) cm.$^{-1}$, n.m.r.: 1·02 (angular CH_3), 2·06 (OCO·CH_3), 3·68 (CO_2CH_3), 5·1 (3α-H), 5·89 (21-H) δ. A solution of olefin (VIIb) (0·035 g.) in dry dioxan (5 ml.) was heated at reflux with 2,3-dichloro-5,6-dicyanoquinone (0·03 g.)[8] for 20 hr. After dilution with methylene chloride, the solution was passed through a column of neutral (Merck) alumina to give 0·02 g. of semi-solid which on crystallization from acetone–methanol yielded 3β-acetoxyisobufalin methyl ester (IIb), m.p. 172–174°. An authentic specimen prepared from bufalin melted at 171—173° and displayed rotatory dispersion: 588 (—82·66°), 500 (—148·7°), 450 (—252·0°), 400 (—403·0°), 350 (—1219·0°) mμ, λ_{max}

293 mμ (ε 27,120), i.r.: ($CHCl_3$) 1730 (COMe), 1720 (CO_2Me), 1603 (C=C–O–), 1265 (OCO·Me), 1165 (C–O) cm.$^{-1}$; n.m.r.: 1·0 (angular CH_3), 2·05 (OCO·CH_3), 3·73 (CO_2CH_3), 5·09 (3α-H), 5·63 (23-H; J 15 c./sec.),6·59 (21-H), 7·23 (22-H; J 15 c./sec.) δ. The sample of ester (IIb) prepared from digitoxigenin exhibited identical o.r.d., u.v., i.r., and n.m.r. spectra. The melting point remained undepressed on admixture with methyl ester (IIb) obtained from bufalin. Experiments directed at reversing the bufalin → 3β-acetoxyisobufalin methyl ester transformation are currently in progress.

(*Received, May 8th*, 1967; *Com.* 441.)

[1] J. C. Knight, G. R. Pettit, and J. Belletire, *J. Chem. Soc.* (*C*), 1967, to be published.
[2] V. Deulofeu, "Progress in the Chemistry of Organic Natural Products," vol. 5, ed. L. Zechmeister, Springer Verlag, 1948, pp. 241—266; E. B. Buckley and N. Porges, Publication No. 44, American Association for the Advancement of Science, Berkeley, California, Dec. 1954, p. 75.
[3] A. von Wartburg and J. Renz, *Helv. Chim. Acta*, 1959, **42**, 1620; K. Meyer, *Experientia*, 1948, **4**, 385.
[4] K. Meyer, *Pharm. Acta Helv.*, 1949, **24**, 222; K. Meyer, *Helv. Chim. Acta.*, 1949, **32**, 1238.
[5] N. Danieli, Y. Mazur, and F. Sondheimer, *Tetrahedron*, 1966, **22**, 3189; F. Sondheimer, *Chem. in Britain*, 1965, **1**, 454.
[6] T. R. Kasturi, G. R. Pettit, and J. Occolowitz, *Chem. Comm.*, 1967, 334.
[7] M. S. Newman and S. Otsuka, *J. Org. Chem.*, 1958, **23**, 797.
[8] B. Berkoz, L. Cuéllar, R. Grezemkovsky, N. V. Avila, and A. D. Cross, *Proc. Chem. Soc.*, 1964, 215; for leading references to DDQ dehydrogenation see also: H.-D. Becker, *J. Org. Chem.*, 1965, **30**, 982; 989; and D. Walker and J. D Hiebert, *Chem. Rev.*, 1967, **67**, 153.