(4) R. N. Icke, B. B. Wisegarver, and G. A. Alles, *Org. Syn.*, **25**, 89(1945).
(5) H. E. Baumgarten, *J. Amer. Chem. Soc.*, **84**, 4975(1962).
(6) J. C. Sheehan and I. Lengyel, *ibid.*, **86**, 1356(1964).
(7) J. C. Sheehan and J. J. Beeson, *ibid.*, **89**, 362(1967).
(8) H. E. Baumgarten, *ibid.*, **85**, 3303(1963).
(9) R. J. Wurtman and J. Axelrod, *J. Biochem. Pharmacol.*, **12**, 1439(1963).
(10) J. C. Towne, *Nature*, **201**, 709(1964).
(11) P. Truitt, E. E. Richardson, L. N. Long, and W. J. Middleton, *J. Amer. Chem. Soc.*, **71**, 3479(1949).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 8, 1969, from the *Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907*

Accepted for publication October 10, 1969.

Abstracted in part from a thesis submitted by J. K. Shields in partial fulfillment of Master of Science degree requirements.

* NDEA Title IV Fellow.

Some Cyclic Ketals and Acetals of Digitoxin, Digoxin, and Ouabain

KHALID S. ISHAQ and OLE GISVOLD

Abstract □ A new and milder method has been utilized to reinvestigate the preparation, properties, and biological activities of the mono-*o*-isopropylidene (acetonides) derivatives of digitoxin, digoxin, and ouabain. The preparation, properties, and biological activities of the mono-*o*-ethylidene derivatives of digitoxin and digoxin also are described. The latter derivatives show greater activity than the former.

Keyphrases □ Digitoxin, digoxin cyclic ketals, acetals—synthesis □ Ouabain cyclic ketals, acetals—synthesis □ Bioanalysis—digitoxin, digoxin, ouabain derivatives □ TLC, paper chromatography—separation, identification □ UV spectrophotometry—identity

In a previous publication the preparation and activities of the mono-*o*-isopropylidene derivatives (glycoside acetonides) of digitoxin, digoxin, and ouabain have been reported (1). Their biological activities were much less than had been expected on the basis of their physical properties and, in the cases of digitoxin and of digoxin, the position of the isopropylidene group. Because these activities were so unexpectedly low, a reinvestigation of the preparation of these derivatives by much milder methods was sought and one such was found. Acetonation can be effected in a very short time at room temperature *via* the use of 2,2-dimethoxypropane, acetone, a trace of acid, and room temperature conditions. The yields are almost quantitative and derivatives readily can be crystallized. Bioassay of these derivatives prepared by this new, very mild method gave comparable results in the case of digoxin but not in the case of digitoxin or ouabain. The ease with which the isopropylidene derivatives (dioxolanes) of digitoxin and digoxin could be prepared *via* the use of ketal-interchange techniques suggested the preparation of other cyclic ketals and cyclic acetals (dioxolanes) to test further the contribution to activity such derivatives might exert. Because the isopropylidene group, even though it was the simplest type of a cyclic ketal and on the terminal digitoxose residue, reduced activity so markedly that the preparation of a cyclic acetal as a second type of a dioxolane derivative was investigated. The ethylidene derivative readily could be prepared *via* 1,1-dimethoxyethane, acetaldehyde, a trace of acid, and room temperature conditions. Bioassays of these ethylidene derivatives *via* LD_{50} intravenous assays in cats showed that mono-*o*-ethylidene digoxin had the same activity as that of acetyldigoxin whereas acetyldigitoxin was 2.35 times as active as mono-*o*-ethylidene digitoxin. Thus the activity (toxicity) of digitoxin was reduced to a greater extent by both types of derivatives than was the case with digoxin. Also it is of interest to note that the mono-*o*-ethylidene digoxin activity was the same as that of acetyldigoxin.

In the case of digoxin, its usefulness is improved in its acetyl derivatives.[1] Acetyldigoxin is considered by some to be the drug of choice because it is well absorbed orally, is less toxic to the CNS, and has a shorter duration of action than digitoxin or acetyldigitoxin. Acetyldigoxin was isolated directly from *Digitalis lanata* by Hopponen and Gisvold (2) in 1952, and its good oral absorption properties in cats were first reported by White and Gisvold (3).

It now appears that the physical properties of the monoethylidene derivative of digoxin, *i.e.*, R_f value measuring liposolubility, should enhance its degree of absorption upon oral administration. Its toxicity and duration of action would depend in part upon its distribution and metabolism *in vivo*. This derivative is sensitive to hydrolysis at condition below pH 7 but is quite stable above pH 7. Dioxolanes are usually stable *in vivo* and thus such derivatives of the cardiac glycosides might exert some interesting effects upon their metabolism *in vivo*. Increased stability without a significant increase in stereochemical bulk would be effected by the substitution of F for H in the ethylidene residue. Such investigations are now in progress.

EXPERIMENTAL

The details of some of the paper chromatographic techniques used in these studies have been previously described (4). Thin-layer

[1] Marketed in Europe as Novadigal and Lanatilin.

chromatography (5) using silica gel and development with benzene–95% ethyl alcohol was used to good advantage. The Raymond reagent was used to detect the glycosides and their reaction products on both paper and silica gel.

Preparation of Isopropylidene Derivatives—The dry glycosides, digoxin, digitoxin, or ouabain (65 mg. each), were dissolved in 130, 65, and 60 ml., respectively, of dried acetone followed by the addition of 15 ml. of 2,2-dimethoxypropane. Dry hydrogen chloride, 12–14 bubbles, was added *via* a glass tube that had a 4-mm. internal diameter. The course of the reaction could be followed by the chromatographic examination of a few milliliters of the reaction mixture. At room temperature, 95 min., 90 min., and 27 hr., respectively, gave the optimum conversion to the desired derivative which was at least 90% in the case of digoxin and digitoxin and 80% plus in the case of ouabain. The remainder was the glycoside and a faster moving substance on TLC silica gel. Hydrogen chloride was chosen as the condensing agent because it readily could be removed under a vacuum on the Buchi during the removal of the acetone and excess 2,2-dimethoxypropane. This is a critical factor since nonvolatile acids such as *p*-toluene sulfonic acid, although effective as a catalyst, are troublesome to remove and may lead to some cleavage of the acid-sensitive dioxolane derivatives.

Although conversion to the desired isopropylidene derivative was obtained in very high yield, preparation of a highly purified analytically pure sample *via* direct crystallization was not readily possible because of the formation of isomorphous crystals. Chromatographic purification on 1-mm. silica gel plates using benzene–95% ethyl alcohol (7:3) yielded analytically pure samples. Such samples gave a positive Raymond test, negative periodate-benzidine test (6), and showed a UV $\lambda_{max.}$ at 218 mμ. Thus the lactone ring was intact and the isopropylidene residue was on the terminal digitoxose residue. The R_f value of these analytically pure samples was the same as that obtained from the initial acetonated glycoside product.

Mono-*o*-isopropylidene digoxin after recrystallization from benzene and naphtha (Skellysolve B) melted at 241–244.5° with decomposition.

Anal.—Calcd. for mono-*o*-isopropylidene digoxin, $C_{44}H_{68}O_{14}$: C, 64.36; H, 8.34. Found: C, 64.13; H, 8.54.

Mono-*o*-isopropylidene digitoxin after recrystallization from benzene and naphtha melted at 243.5–246.5° with decomposition.

Anal.—Calcd. for mono-*o*-isopropylidene digitoxin, $C_{44}H_{68}O_{13}$: C, 65.65; H, 8.50. Found: C, 65.43; H, 8.36.

Mono-*o*-isopropylidene ouabain after recrystallization from methanol, benzene, and naphtha melted at 221–223° with decomposition.

Anal.—Calcd. for mono-*o*-isopropylidene ouabain, $C_{32}H_{48}O_{12}$: C, 61.55; H, 7.74. Found: C, 61.50; H, 7.96.

Preparation of Ethylidene Derivatives—Dry digoxin, 80 mg., was dissolved with the aid of heat in 170 ml. of dried methylene chloride in a 250-ml. three-necked flask fitted with a condenser equipped with a drying tube. The flask was cooled to 5° in an ice bath and 5 ml. of 1,1-dimethoxyethane added. Dried and cooled acetaldehyde, 10 ml., was added *via* a syringe and finally dry hydrogen chloride, 33–55 bubbles, was introduced *via* a glass tube having an internal diameter of 4 mm. After 17 hr. the solvents were removed under vacuum on the Buchi. Analysis *via* TLC on silica gel using benzene–95% ethyl alcohol (7:3) showed the presence of some digoxin R_f value 0.50 and two very closely moving substances having an average R_f value of 0.67. The latter might well be two conformers or isomers at the 2-position of the dioxolane ring. Purification *via* thick-layer silica gel plates as described above gave a crystalline product after crystallization from methanol, benzene, and water that melted at 128–148°; $[\alpha]_D^{22}$ = +9.6 (C = 0.5 in methanol); $\lambda_{max.}$ at 218 mμ.

Anal.—Calcd. for mono-*o*-ethylidene digoxin, $C_{44}H_{66}O_{14}$: C, 63.99; H, 8.24. Found: C, 63.44; H, 8.53.

Because digitoxin was more soluble in methylene chloride, 80 mg. was dissolved in 40 ml. of methylene chloride and a 200-ml. three-necked flask was used. The remainder of the experimental conditions were the same as those used for the preparation of mono-*o*-ethylidene digoxin. The purified mono-*o*-ethylidene digitoxin was crystallized from methanol, benzene, and water. It melted at 113–127°, $[\alpha]_D^{22}$ = + 8.5 (C = 0.5 in methanol); $\lambda_{max.}$ at 218 mμ. Its R_f value on silica gel using benzene–95% ethyl alcohol (7:3) was 0.72. As in the case of the digoxin derivative, two very closely moving spots were obtained. Thus here also two conformers or isomers at the 2-position of the dioxolane ring are possible to account for the two very closely moving spots on TLC since the R_f value of digitoxin was 0.57.

Anal.—Calcd. for mono-*o*-ethylidene digitoxin, $C_{43}H_{66}O_{13}$: C, 65.29; H, 8.41. Found: C, 64.73; H, 8.62.

Bioassay[2] of the Glycoside Acetonides—The following mean LD_{50} ± *SE* mg./kg. was obtained: digoxin acetonide, 0.582 ± 0.04 (4 cats); digitoxin acetonide, 1.38 ± 0.03 (4 cats); ouabain acetonide, no minimum lethal dose could be reached (2 cats). Thus the digoxin acetonide was slightly more active than that previously reported whereas digitoxin acetonide was about 1.8 times as active as that previously reported. The inactivity of the ouabain acetonide differed from that previously reported which was 0.8837 ± 0.0714 (10 cats).

Bioassay[2] of the Glycoside Mono-*o*-ethylidene Derivatives—The following mean LD_{50} ± *SE* mg./kg. was obtained: mono-*o*-ethylidene digoxin, 0.375 ± 0.02 (4 cats), and mono-*o*-ethylidene digitoxin, 1.05 ± 0.06.

REFERENCES

(1) O. Gisvold, *J. Pharm. Sci.*, **55,** 1350(1966).

(2) R. Hopponen and O. Gisvold, *J. Amer. Pharm. Ass., Sci. Ed.*, **41,** 146(1952).

(3) W. F. White and O. Gisvold, *ibid.*, **41,** 42(1952).

(4) O. Gisvold, *ibid.*, **47,** 594(1958); **47,** 600(1958); O. Gisvold and S. E. Wright, *ibid.*, **46,** 535(1957).

(5) E. J. Johnston, *J. Pharm. Sci.*, **55,** 531(1966).

(6) J. M. Clark, Jr., "Experimental Biochemistry," Freeman, San Francisco, Calif., 1964, p. 213.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 21, 1969, from the *College of Pharmacy, Department of Medical Chemistry, University of Minnesota, Minneapolis, MN 55455*

Accepted for publication September 25, 1969.

This investigation was supported in part by research grant HE-06569 from the National Heart Institute, U. S. Public Health Service, Bethesda, Md.

[2] The authors wish to acknowledge the assistance of Dr. W. F. White and Dr. S. A. El-Refrawy in performing the bioassays of these derivatives.