(4) Baker, B. R., and Ho, B.-T., *J. Heterocyclic Chem.*, **2**, 335(1965).
(5) Baker, B. R., and Lourens, G. J., *ibid.*, **2**, 344(1965).
(6) Baker, B. R., Schwan, T. J., Novotny, J., and Ho, B.-T., *J. Pharm. Sci.*, **55**, 295(1966).
(7) Baker, B. R., Ho, B.-T., Coward, J. K., and Santi, D. V., *ibid.*, **55**, 302(1966).
(8) Baker, B. R., and Shapiro, H. S., *ibid.*, **55**, 308(1966).
(9) Baker, B. R., and Jordaan, J. H., *J. Heterocyclic Chem.*, **2**, 162(1965).
(10) Baker, B. R., and Jordaan, J. H., *J. Pharm. Sci.*, **54**, 1740(1965).
(11) *Ibid.*, **55**, 1417(1966).
(12) Baker, B. R., Ho, B.-T., and Neilson, T., *J. Heterocyclic Chem.*, **1**, 79(1964).
(13) Baker, B. R., *et al.*, *J. Am. Chem. Soc.*, **83**, 3713(1961).
(14) Baker, B. R., Lee, W. W., and Tong, E., *J. Theoret. Biol.*, **3**, 459(1962).
(15) Baker, B. R., "Design of Active-Site-Directed Irreversible Ezyme Inhibitors. The Organic Chemistry of the Enzymic Active Site," John Wiley & Sons, Inc., New York, N. Y., to be published.
(16) Baker, B. R., *et al.*, *J. Heterocyclic Chem.*, to be published.
(17) Baker, B. R., Santi, D. V., and Shapiro, H. S., *J. Pharm. Sci.*, **53**, 1317(1964).
(18) Gagnon, P. G., Nadeau, G., and Côté, R., *Can. J. Chem.*, **30**, 592(1952).
(19) Baker, B. R., and Shapiro, H. S., *J. Med. Chem.*, 6 664(1963).
(20) Baker, B. R., and Santi, D. V., unpublished data.

Chemistry and Pharmacology of a Glycoside of *Vallaris solanacea*

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One of the glycosides of *Vallaris solanacea* has been identified as *O*-acetyl-solanoside (*O*-acetyl acofreosyl-digitoxigenin). It possesses potent cardiotonic activity. Its pharmacological properties have been compared with those of lanatoside C and digoxin.

In a previous communication (1) it was reported that the glycoside mixture obtained from the leaves of *Vallaris solanacea* Kuntze (N.O. *Apocynaceae*) possesses powerful digitalis-like activity. Two recent publications by Kaufmann *et al.* (2, 3), describing the isolation and determination of the structure of six new glycosides from the seeds of *V. solanacea*, have prompted the authors to report the observation of glycoside B, one of the glycosides obtained from the leaves.

EXPERIMENTAL

Chemistry

Shade-dried leaves of *V. solanacea*, collected from Kashmir, India, were percolated with ethanol and the percolate was concentrated to about one-fifth its volume. The concentrate was diluted with an equal volume of water and extracted with benzene. The aqueous phase was concentrated *in vacuo* and extracted with chloroform. The residue from the chloroform extract was taken up in chloroform and chromatographed on a column of silica gel (E. Merck, fine grade), and the column developed with chloroform containing increasing proportions of methanol when three major glycoside fractions A, B, and C were obtained. On thin-layer chromatography [Silica Gel G; solvent system, ethyl methyl ketone–cyclohexane (1:1)] fractions A and C showed up as two spots, while fraction B gave only one spot (spots detected by spraying with water). Preparative thin-layer chromatography of fraction B [Silica Gel G; solvent system, chloroform–isopropyl alcohol (19:1)] gave glycoside B, which was crystallized from chloroform–hexane and ethanol–water mixture, m.p. 137–139° (Kofler block). The angle of rotation was $[\alpha]_D^{20} - 16$ (±2) (c, 1 in methanol). The homogeneity of the glycoside was established by thin-layer chromatography (Silica Gel G) in four solvent systems: ethyl acetate; chloroform–isopropyl alcohol (19:1); ethyl methyl ketone–cyclohexane (1:1); benzene methanol (3:1).

Anal.—Calcd. for $C_{32}H_{48}O_9$: C, 66.6; H, 8.39. Found: C, 67.05; H, 8.75.

Mannich hydrolysis of glycoside B gave a single sugar which was identified as acofreose by paper chromatographic comparison with an authentic sample [solvent systems: methyl ethyl ketone–*n*-butanol (1:1)/borate buffer; toluene–*n*-butanol (1:1)/water], while Killiani hydrolysis yielded digitoxigenin. The highly nonpolar character of glycoside B suggested the possibility of one of its hydroxyl groups being blocked. Its I.R. absorption spectrum and color reactions indicated its identity with *O*-acetyl-acofreoside of digitoxigenin (*O*-acetyl-solanoside) (3). This was confirmed by comparison with an authentic sample of *O*-acetyl-solanoside using thin-layer chromatography as described above.

A thin-layer chromatographic comparison kindly carried out by Reichstein of fractions A and C with the glycosides obtained from the seeds (2, 3), showed that the glycosides contained in the leaves are very similar to those present in the seeds.

Pharmacological Activity

Materials and Methods.—A stock solution of glycoside B containing 500 mcg./ml. was prepared

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The authors are grateful to Professor T. Reichstein for direct comparison of the glycosides obtained by us with those isolated in their laboratory, to Mr. P. A. George for statistical analysis, to Dr. (Mrs.) S. Nitya Nand for help in ECG studies, and to Dr. C. Ray for his interest in the work.

Fig. 1.—Isolated papillary muscle of cat. Muscle stimulated supramaximally (12 v., 30/min., 1 msec. duration). At arrow glycoside B (10 mcg./ml.) was added to the bath. Key: 1, normal amplitude of contraction; 2, typodynamic state; 3, onset of improvement in the amplitude of contraction after 8 min. of addition of glycoside B; 4, complete restoration of the amplitude of contraction 40 min. after the addition of the glycoside; 5, decrease in the amplitude of contraction 60 min. after the addition.

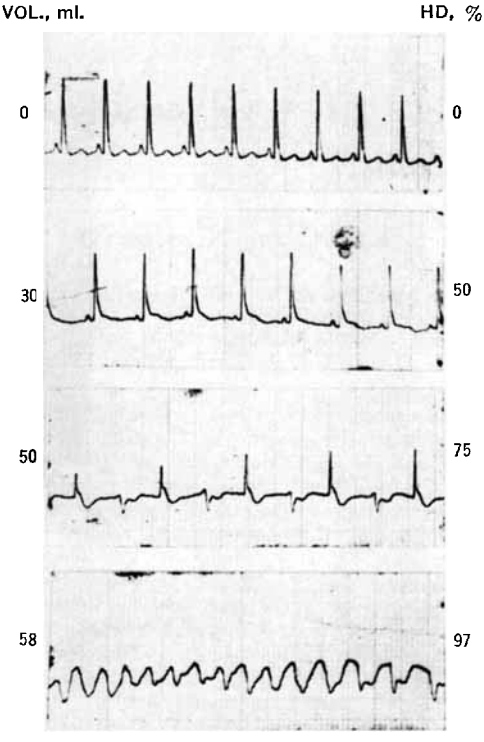

Fig. 2.—Electrocardiographic changes in an anesthetized cat (3.2 Kg.) recorded on standard lead II. Glycoside B was perfused (i.v.) at the rate of 20 mcg./ml./min. Key: 0 ml., normal record, sinus rhythm, T-wave upright, heart rate 210/min.; 30 ml., sinus rhythm, reverted biphasic T-wave, heart rate 180/min.; 50 ml., irregular ventricular extra systoles; 58 ml., ventricular tachycardia and fibrillation. Numbers on the right side denote the percentage of Hatcher dose against volume perfused shown on the left side.

by dissolving 10 mg. in 1 ml. of 90% alcohol and diluting with 19 ml. of normal saline. Further dilutions were made as required just before the experiment.

Cardiotonic Activity.—Direct proof of the cardiotonic activity of glycoside B was obtained by (*a*) perfusing the frog heart (*Rana tigrina*) through the inferior vena cava, (*b*) perfusing isolated hypodynamic guinea pig heart, prepared according to the method of Vick and Kehn (4), and (*c*) studying its action on the hypodynamic papillary muscle of cats (5).

Indirect evidence of the cardiotonic activity of the glycoside was obtained by (*a*) recording electrocardiographic (ECG) changes on standard lead II in anesthetized cats following continuous perfusion, as well as after single 10–50% of Hatcher dose (HD), (*b*) studying its effects on guinea pig or rabbit ileum *in vitro* and on intestinal movements of anesthetized cats.

Intensity of Biological Activity.—This was determined in chloralosed cats (80 mg./Kg. i.v.) by infusing continuously a solution of 25 mcg./ml./min. The concentration was so adjusted as to cause cardiac arrest within 50–60 min. of perfusion. The British Pharmacopoeia (1958) method was employed for the assay in guinea pigs. A concentration of 50 mcg./ml. of glycoside B was used which brought about cardiac arrest in 20–40 min.

Absorption, Persistence, and Cumulative Toxicity.—Its absorption from the gastrointestinal tract after oral feeding was measured in unanesthetized cats by the method of Purdum (6), and in anesthetized cats by the method of Dille and Whatmore (7). The persistence of the glycoside was studied in cats after intravenous administration of 40% HD. Cumulative toxicity was determined by the method described earlier (8) by daily subcutaneous injections of 20 and 40% HD for 21 days, or less if death took place earlier. For comparative purposes digoxin and lanatoside C were used in most of these studies.

RESULTS

Cardiotonic Activity.—*Direct Evidence.*—(*a*) Perfusion of the frog heart with glycoside B in concentrations of 5–30 mcg./ml. produced an initial increase in the amplitude of contraction and a slowing of the heart rate. This was followed by partial A-V block and systolic standstill of the heart.

(*b*) Perfusion of the hypodynamic guinea pig heart with 5 and 10 mcg./ml. concentrations of glycoside B produced a marked positive inotropic effect within 5–7 min. Further perfusion resulted in cardiac irregularities followed by cardiac arrest in 15–20 min.

(*c*) Glycoside B, in a concentration of 0.4–0.5 mcg./ml., restored the contractility of the hypodynamic papillary muscle of the cat. At low concentrations (0.04–0.1 mcg./ml.) improvement in the amplitude of contraction appeared within 5–7 min. of addition, the prefatigue amplitude being attained in 20–30 min. and being well maintained for about 2 hr. At high concentration (0.5 mcg./ml.) the restored contractility lasted for 45–60 min.; there-

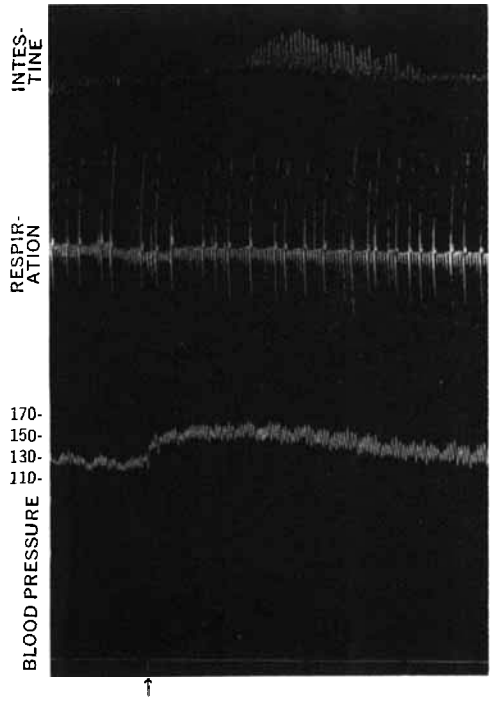

Fig. 3.—The effect of 25% Hatcher dose (arrow) of glycoside B in an anesthetized cat.

TABLE I.—MEAN LETHAL DOSES OF GLYCOSIDE B, LANATOSIDE C, AND DIGOXIN IN CATS AND GUINEA PIGS

Drug	Mean Lethal Dose (mcg./Kg.) Mean ± S.E. Cat	Guinea Pig
Glycoside B	388.2 ± 10.85 (8)[a]	1093.0 ± 29.05 (6)
Lanatoside C	200.2 ± 8.75 (5)	483.9 ± 22.00 (6)
Digoxin	278.0 ± 7.56 (4)	838.17 ± 31.67 (7)

[a] Figures in parentheses indicate the number of animals used.

after, the amplitude of contraction declined steadily and ultimately the muscle became unresponsive to the stimuli (Fig. 1). Occasionally, this concentration increased the irritability of the preparation.

Indirect Evidence.—(*a*) The ECG record following continuous infusion with glycoside B showed bradycardia, prolongation of PR interval, sagging of ST segment, PR dissociation, ventricular extra systoles followed by irregular ventricular rhythm, and cardiac standstill (Fig. 2), typical effects of a cardenolide.

The therapeutic index of glycoside B was calculated from the ECG records. Bradycardia, prolongation of PR interval, inversion of T wave, or sagging of ST segment were considered as the therapeutic stage, the appearance of extra systoles and bundle block as the early toxic stages, and ventricular tachycardia and fibrillation as the severe toxic stage. Results showed that 25–50% of the lethal dose was required to produce the therapeutic stage while 60–90% of the same produced the severe toxic stage. In comparative studies with ouabain (10 mcg./ml.) these changes were produced with 30–35 and 55–75% of the lethal dose, respectively.

A study of the effect of single intravenous doses of glycoside B on the ECG changes in anesthetized cats revealed that 10 and 25% HD produced prolongation of PR from 0.066 to 0.0825 sec., 10–14% bradycardia, and slight increase in QRS within 5–10 min. of administration; change in the T wave was also noticed. On the other hand 50% HD produced inversion of T wave and sagging of ST segment followed by ventricular tachycardia after 25 min. and death after 40 min.

(*b*) In pigeons, intravenous doses of 100, 150, 200, and 300 mcg./Kg. of glycoside B produced 16, 42, 71, and 100% emesis, respectively. The lag period decreased as the dose was increased.

(*c*) Glycoside B in a concentration of 3×10^{-5} produced a marked contraction of isolated segments of guinea pig ileum and increased the tone and amplitude of contraction of isolated rabbit ileum.

Intravenous administration of 25% HD of glycoside B in anesthetized cats produced a slight rise in blood pressure and an increase in the tone and

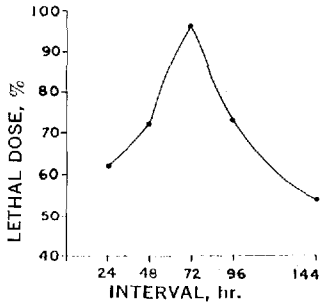

Fig. 4.—Persistence of 40% Hatcher dose of glycoside B administered intravenously denoting time in hours at which the animals were perfused to determine the persistence and the percentage of lethal dose needed to kill the treated animals

TABLE II.—ABSORPTION IN UNANESTHETIZED CATS AFTER 5 hr. AND IN ANESTHETIZED CATS AFTER 1 hr. FOLLOWING THE ADMINISTRATION OF 1 HATCHER DOSE OF GLYCOSIDE B, LANATOSIDE C, AND DIGOXIN

Drug	Hatcher Doses in Unanesthetized Cats (mcg./Kg.) ± S.E. Control	Treated	% Absorption	Hatcher Doses in Anesthetized Cats (mcg./Kg.) ± S.E. Control	Treated	% Absorption
Glycoside B	288.20 ± 6.85 (8)[a]	140.00 ± 4.46 (7)	51.3	329.40 ± 3.87 (3)	0.00 (6)	100.00
Lanatoside C	200.20 ± 8.75 (5)	169.10 ± 2.05 (5)	15.2	183.70 ± 2.50 (3)	157.20 ± 3.25 (3)	14.42
Digoxin	...	...	...	254.20 ± 2.30 (2)	180.00 ± 5.02 (2)	29.13

[a] Figures in parentheses indicate the number of animals used.

TABLE III.—CUMULATIVE TOXICITY OF GLYCOSIDE B, LANATOSIDE C, AND DIGOXIN

Drug	Cats, No.	Hatcher Dose Administration Subcutaneously, %	Deaths, No.	Av. Day of Death	Observations
Lanatoside C	3	20	3/3	12	1 died on 9th day, 1 on 11th day, and 1 on 15th day
		40	3/3	7	2 died on 7th day, and 2 on 9th day
Glycoside B	3	20	2/3	8	2 died on 8th day
	5	40	5/5	4	2 died on 2nd day, 1 on 3rd day, 1 on 5th day, and 1 on 9th day
Digoxin	2	20	2/2	6	1 died on 5th day, and 1 on 7th day

amplitude of contraction of the intestines. The latter effect lasted for 5–10 min. (Fig. 3).

Intensity of Biological Activity.—Table I shows that glycoside B is more active in cats than in guinea pigs, that its potency is three-fourths that of digoxin and one-half that of lanatoside C, both in cats and guinea pigs.

Absorption.—The comparative absorption of glycoside B and of lanatoside C was studied in unanesthetized cats following oral administration of one HD of each. The results, calculated in terms of the absolute absorption, are shown in Table II. Glycoside B was found to be very well absorbed (63.9%) from the gastrointestinal tract, while the absorption of lanatoside C was only 15.2%. Furthermore, the oral absorption of the former was found to be consistent and dependable since it varied only from 52.9 to 70.0% in a series of six animals.

The absorption of the glycoside from the small intestine was studied in five anesthetized cats by administering one HD to the intestine isolated between two ligatures. All animals died between 20–55 min. after administration, indicating that the main site of absorption is the intestine. In these experiments lanatoside C and digoxin showed 14.4 and 29.13% absorption, respectively.

Persistence.—The persistence of glycoside B showed a biphasic phenomenon (Fig. 4). At 72 hr. the glycoside appeared to have been completely eliminated from the system as shown by the percentage of HD required to kill the treated animals. However, after 96 hr. this dose decreased, so much so that at 144 hr. the dose was even lower than that required at the beginning of the experiment. The implication of this observation is discussed later.

Cumulative Toxicity.—The results are shown in Table III along with those obtained with lanatoside C and digoxin. The average number of days taken for the animals to die indicate that the cumulative toxicity of glycoside B lies between that of digoxin and lanatoside C. All the treated animals showed marked emaciation, apparently due to loss of appetite and body weight.

DISCUSSION

Glycoside B obtained from the leaves of *V. solanacea* has been identified as *O*-acetyl-solanoside which is present in the seeds.

Glycoside B is found to possess marked cardiotonic activity which is one-half that of digoxin and one-half and one-fourth that of lanatoside C in cats and guinea pigs, respectively. Its therapeutic index is the same as that of ouabain. It has a quick onset of action like that of ouabain and lanatoside C which may be an advantage over digoxin. This may be attributed to its highly nonpolar character. Its good, consistent, and dependable absorption after oral administration resembles digoxin more than lanatoside C.

The fact that administration of one HD into the intestine invariably produced cardiac standstill within 20–25 min., while the same dose given orally failed to produce cardiac arrest within 6 hr. would indicate that glycoside B is inactivated to some extent by the gastric juices.

In persistence studies glycoside B has been found to possess medium duration of action as the effect of a single dose lasts for 72 hr. In this respect it resembles lanatoside C more than digoxin. However, a peculiar behavior of glycoside B has been observed. At 72 hr. the effect of a single dose was found to have disappeared, but observations carried out at 96 and 144 hr. indicated the reappearance of the effect so much so that at the latter interval it was even greater than the effect produced initially when the glycoside was administered. Obviously, this phenomenon cannot be explained on the basis of the actual reappearance of the glycoside B itself in the system, but must be due to the sensitization of the receptors to the action of the drug so that at 96 and 144 hr. a lesser dose of the glycoside can produce the same effect. Rothlin (9) has reported the phenomenon of sensitization and desensitization, tachyphylaxis, and tolerance with digoxin, digilanid A, and digilanid C in cats. The observation regarding the medium duration of action of glycoside B is also supported by the cumulative toxicity data which show that it possesses a low toxicity compared to digoxin but a high value as compared to lanatoside C.

It may be concluded that glycoside B is a potent cardenolide with a therapeutic index similar to that of the cardiac glycosides used clinically. It has a quick onset, medium duration of action, and shows consistent and dependable oral absorption with low cumulative toxicity.

REFERENCES

(1) Vohra, M. M., and De, N. N., *J. Sci. Ind. Res.*, **20C**, 161(1961).
(2) Kaufmann, H., Wehrli, W., and Reichstein, T., *Helv. Clin. Acta*, **48**, 65(1965).
(3) Kaufmann, H., *ibid.*, **48**, 83(1965).
(4) Vick, R. L., and Kehn, J. B., Jr., *J. Pharmacol. Exptl. Therap.*, **121**, 389(1957).
(5) Vohra, M. M., and De, N. N., *Ind. J. Med. Res.*, **51**, 937(1963).
(6) Purdum, W. A., *J. Am. Pharm. Assoc.*, **26**, 17(1937).
(7) Dille, J. M., and Whatmore, G. B., *J. Pharmacol. Exptl. Therap.*, **75**, 350(1942).
(8) Vohra, M. M., Kohli, J. D., and De, N. N., *Arch. Intern. Pharmacodyn.*, **133**, 265(1961).
(9) Rothlin, E., *Bull. Schweiz. Chem. Akad. Med. Wiss.*, **4**, 379(1948).