[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF THE CITY COLLEGE OF NEW YORK, COLUMBIA UNIVERSITY, AND THE UNIVERSITY OF MICHIGAN]

Amino Derivatives of Strophanthidin. I. Reactions of Primary and Secondary Amines with the Butenolide Side Chain of Strophanthidin[1]

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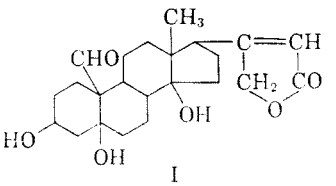
Strophanthidin undergoes aminolysis of the butenolide side-chain when heated with primary amines to give $\Delta^{\alpha,\beta}$-unsaturated γ-lactams. Further reaction with additional amine leads to more complicated products. With secondary amines, the butenolide side-chain of strophanthidin apparently is converted to an eneamine-amide. The structures of these substances are discussed and the reaction of β-phenyl-$\Delta^{\alpha,\beta}$-butenolide with amines has been investigated.

During the attempted preparation of certain 3-(*N*,*N*-dialkyl)glycyl esters of strophanthidin[3] by the reaction of chloroacetylstrophanthidin with secondary amines, nitrogenous products which were evidently mixtures of several bases were obtained. This introduced the possibility that these substances may have arisen from the reaction of the amines with other reactive parts of the strophanthidin molecule (I) possibly not involving the chloroacetyl group at all. As in the preparation of the piperidylglycyl ester of strophanthidin the most complicated mixture of bases was obtained, the direct reaction of strophanthidin with piperidine was investigated. Strophanthidin does indeed react with piperidine to give a mixture of nitrogenous bases. Further, nitrogenous compounds result from the reaction of strophanthidin with primary amines.

The products of the reaction of strophanthidin with amines present interesting possibilities from the standpoint of physiological action.[4] Therefore, an investigation of the nature of the reaction and



I

of the structures of the products was undertaken. In the present paper we present the results of a study of the reaction of strophanthidin with a number of amines both primary and secondary.

The compounds produced by the reaction of one equivalent of strophanthidin with one equivalent of various primary amines are listed in Table I. In general the reactions involved condensation of one equivalent of each of the reactants with elimination of one mole of water. All of the substances gave positive Legal (nitroprusside) tests indicating that the side-chain double bond was still intact. There was strong evidence that the reaction proceeded further with involvement of a second equivalent of amine possibly at other reactive sites of the strophanthidin molecule as mixtures of compounds of higher nitrogen content were found among the reaction products. However, as all attempts to separate these mixtures into pure constituents have failed so far, a discussion of them will be postponed for a later communication. In addition to the amines listed in Table I, the reaction was attempted with ethanolamine, aniline, and tetrahydroisoquinoline. However, the products were too unstable to permit isolation and characterization.

In considering possible structures for the products of the reaction of strophanthidin with one equivalent of a primary amine certain observations have been made. As indicated above all the compounds gave a positive Legal test. Although it was impossible to demonstrate the presence either of the C-10 aldehyde group by formation of carbonyl derivatives of the amine condensation products or the C-3 hydroxyl group by preparation of acyl derivatives, the presence of these functions was indicated indirectly. Strophanthidin benzoate and strophanthidin oxime both reacted with *n*-propylamine in a manner exactly analogous to the reaction of strophanthidin itself with *n*-propylamine. The conclusion appears to be justified that the reaction of strophanthidin with primary amines does not involve either the C-3 hydroxyl group or the C-10 aldehyde group and must be concerned with the butenolide side-chain. From the evidence at hand we believe that the course of this reaction may be represented by I–XI. Further reaction of X with a second equivalent of amine may involve isomerization of X to XII under the influence of the basic amine[5] followed by reaction of the isomerized prod-

(1) This investigation was aided in part by a grant from Eli Lilly and Company to whom we express our appreciation.

(2) Present address: Department of Chemistry, University of Michigan, Ann Arbor, Michigan.

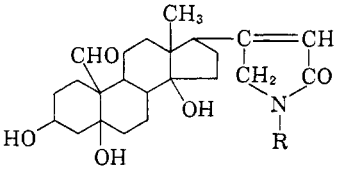
(3) W. Küssner, U.S. patent **2,296,677** (Sept. 22, 1942).

(4) H. L. Otto, T. Greiner, H. Gold, F. Palumbo, L. Warshaw, N. T. Kwit, and K. K. Chen., *J. Pharmacol. and Exp. Therap.*, **107**, 225 (1953). In this paper the structure of the tryptamine derivative of strophanthidin, which was highly uncertain at the time, is erroneously pictured as involving the C-10 aldehyde group.

(5) W. D. Paist, E. R. Blout, F. C. Uhle, and R. C. Elderfield, *J. Org. Chem.*, **6**, 273 (1941).

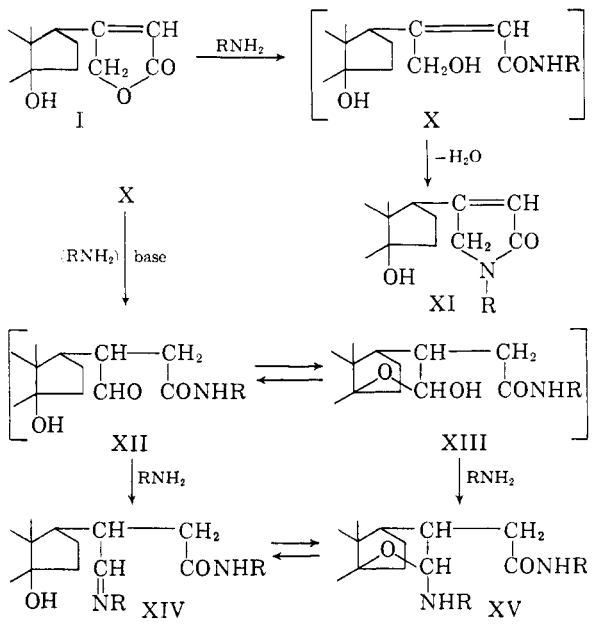
TABLE I

PRODUCTS FROM THE REACTION OF ONE MOLE OF PRIMARY AMINES WITH STROPHANTHIDIN



Reacting Amine		R	Formula	Analysis Calcd. C	H	N	Found C	H	N
n-Propyl	II	*n*-Propyl	$C_{26}H_{39}NO_5$	70.1	8.8	3.1	69.9	9.0	3.1
n-Butyl	III	*n*-Butyl	$C_{27}H_{41}NO_5 \cdot H_2O$	68.8	9.1	3.0	68.6	9.2	2.9
n-Butyl	IV	*n*-Butyl	$C_{27}H_{41}NO_5$[a]	70.6	9.0	3.1	70.9	9.3	3.5
Tryptamine	V	2-(3′-Indolyl)ethyl	$C_{33}H_{42}N_2O_5$	72.5	7.8	5.1	72.5	7.8	5.5
1-Diethylamino-4-aminopentane	VI	4-Diethylamino-1-methylbutyl	$C_{32}H_{52}N_2O_5$	70.6	9.5	5.1	70.6	9.2	4.8
Glycine ethyl ester	VII	Carboethoxymethyl	$C_{27}H_{39}NO_7$	66.5	8.0	2.9	66.4	8.3	2.8
dl-Alanine ethyl ester	VIII	Carboethoxyethyl	$C_{28}H_{41}NO_7$	66.8	8.1	2.8	67.1	8.1	2.9
Tryptophan methyl ester	IX	1-Carbethoxy-2-(3′-indolyl)ethyl	$C_{35}H_{44}N_2O_7$	69.5	7.3	4.6	69.6	7.2	4.5

[a] Anhydrous compound.



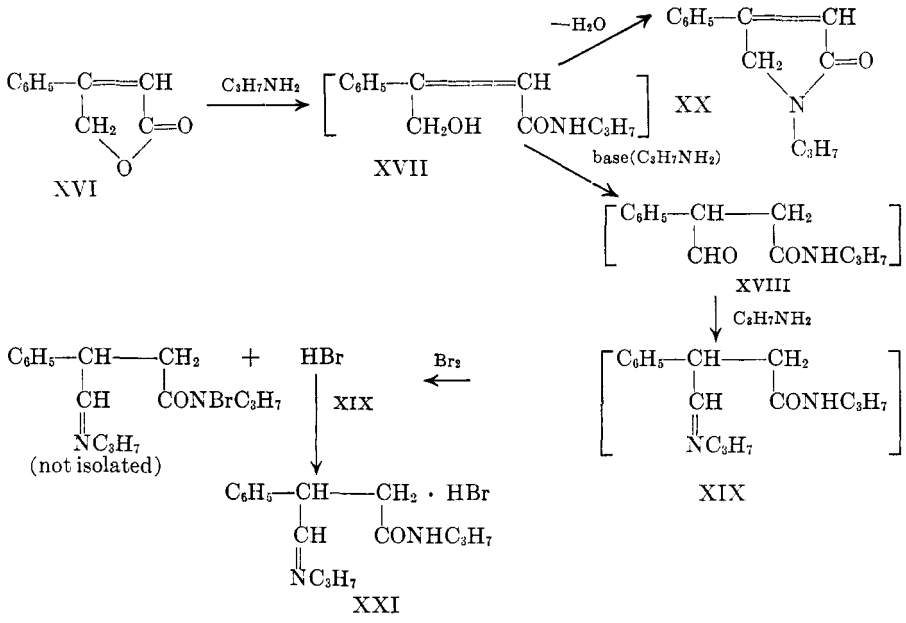
uct with a second equivalent of amine *via* either XII or XIII to yield XIV or XV. As indicated below in connection with the reaction of strophanthidin with secondary amines, we prefer the XII–XIV sequence. In conformity with structure XI the product of the reaction of strophanthidin with *n*-propylamine showed strong infrared absorption characteristic of an *N,N*-disubstituted amide. The compounds were all soluble in dilute hydrochloric acid, a property characteristic of *N,N*-disubstituted amides of sufficiently high molecular weight.[6]

In order to provide confirmation for the above interpretation attention was directed to the reaction of *n*-propylamine with the model compound, β-phenyl-$\Delta^{\alpha,\beta}$-butenolide[7,8] (XVI). This differs from strophanthidin in lacking the C-14 hydroxyl group, the presence of which may play a role in the reactions under consideration, particularly those of strophanthidin with secondary amines (see below). When XVI was allowed to react with *n*-propylamine the reaction took a course which paralleled that of strophanthidin. The initial product of the reaction was the unsaturated alcohol amide (XVII) formed by aminolysis of the butenolide. The greater portion of XVII was isomerized under the influence of the amine to the aldehyde amide (XVIII) which then reacted with a second equivalent of amine to give the imine (XIX), as no hydroxyl group corresponding to the C-14 hydroxyl of strophanthidin was present. A smaller amount of XVII apparently underwent ring closure to give the unsaturated lactam (XX) which was too unstable for purification. However, the crude material gave a positive Legal test. The imine (XIX) was isolated as its hydrobromide when an ethereal solution of it was treated with bromine. Apparently the amide hydrogen was replaced by bromine initially (the analogous amide derived from morpholine which carries no amide hydrogen was inert to bromine) and the hydrogen bromide liberated formed a salt with the unbrominated imine to give the stable hydrobromide (XXI) which was isolated.

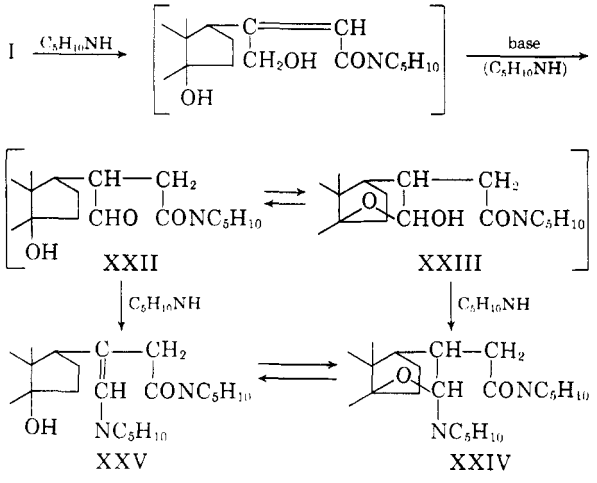
(6) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, *The Systematic Identification of Organic Compounds*, 4th Ed., John Wiley and Sons, New York, 1956, p. 80.

(7) M. Rubin, W. D. Paist, and R. C. Elderfield, *J. Org. Chem.*, **6**, 260 (1941).

(8) R. G. Linville and R. C. Elderfield, *J. Org. Chem.*, **6**, 270 (1941).



The reaction of strophanthidin with two equivalents of a secondary amine—*e.g.*, piperidine or morpholine—paralleled the course suggested above for the reaction with primary amines. However, it was not possible to isolate the product of the reaction of one equivalent of a secondary amine with strophanthidin in pure form. The reaction is formulated as shown in I-XXIV-XXV. In this case



the carbinolamide is incapable of cyclizing through loss of water to give an unsaturated lactam. Hence, it can only undergo isomerization under the influence of the basic amine to give the aldehyde amide (XXII) or its tautomer (XXIII). Reaction of XXII with a second equivalent of secondary amine would then lead to the eneamine (XXV),[9] whereas reaction of XXIII with a second equivalent of secondary amine would lead to XXIV. It is also entirely possible that XXIV and XXV may be in equilibrium.

(9) C. Mannich and H. Davidson, *Ber.*, **69**, 2106 (1936).

Of the above possibilities we favor structure XXV as the most likely possibility based on the infrared spectrum of the substance. Strong absorption bands were present at 1720, 1650, and 1603 cm.$^{-1}$ Of these the band at 1650 cm.$^{-1}$ is the most significant, as Leonard and Gash[10] have shown that such absorption is characteristic of eneamines. Further, the infrared spectrum of the strophanthidin-piperidine product is clear in the region 1000–1200 cm.$^{-1}$ where absorption due to a carbinolamine ether would be expected.[11]

Substantiation of this interpretation was again obtained from a study of the reaction of XVI with morpholine (the products from the reaction with piperidine was exceedingly difficult to manipulate). When XVI was refluxed with morpholine in benzene solution, the product initially obtained was completely soluble in cold dilute hydrochloric acid. Although the substance could not be isolated in pure form, this acid solubility is consistent with either the carbinolamine (XXVI) or eneamine (XXVII) structure both of which would be expected to be reasonably unstable. This assignment was corroborated by conversion of the product to the oxime (XXVIII) during which the nitrogen function was destroyed.

EXPERIMENTAL[12,13]

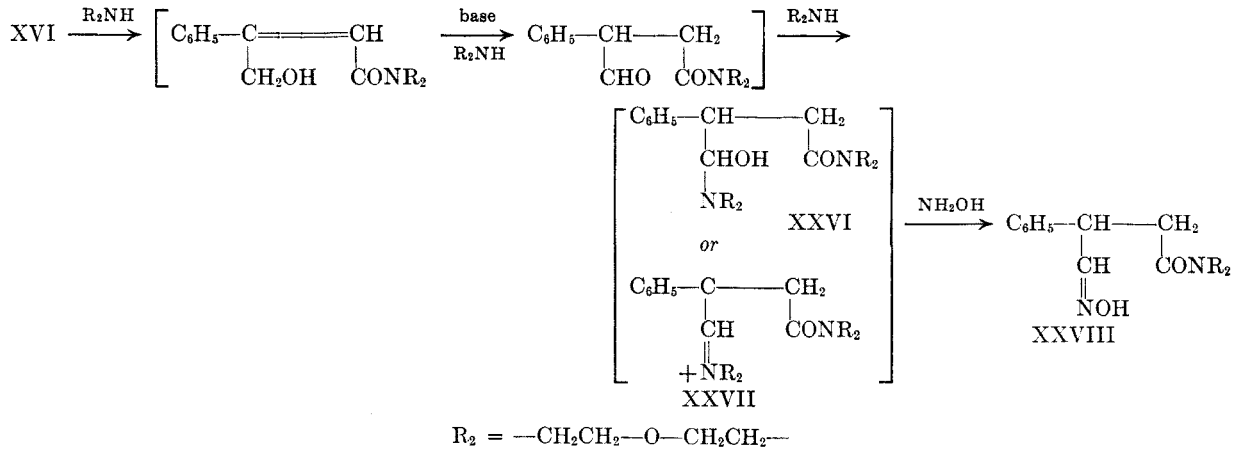
Reaction of strophanthidin with amines. With n-propylamine (II). A mixture of 1 g. of strophanthidin and 10 ml. of redistilled *n*-propylamine was swirled until a clear solution

(10) N. J. Leonard and V. W. Gash, *J. Am. Chem. Soc.*, **76**, 2781 (1954).

(11) Private communication from Dr. O. E. Edwards, National Research Council of Canada, Ottawa, Canada.

(12) All melting points are uncorrected for stem exposure.

(13) Microanalyses by Spang Microanalytical Laboratory, Ann Arbor, Michigan, and Schwartzkopf Microanalytical Laboratory, Woodside, N. Y.



resulted. The solution was boiled in an open flask on the steam bath for 15 min., after which residual traces of the amine were removed at the water pump. The frothy white residue was dissolved in 15 ml. of 3% hydrochloric acid. On addition of 10% sodium carbonate solution a white solid precipitated which was collected, air dried, and taken up in boiling benzene. After filtering from a little insoluble material, addition of pentane to the cooled solution gave a heavy white amorphous precipitate. This was collected, taken up in cold benzene, and chromatographed over an alumina column with 20-ml. portions of 3:1 benzene-absolute methanol as eluent. The material collected in the first three fractions was combined and weighed 499 mg. It contracted at 75°, softened to a glass at 85–90°, and decomposed at 112°. The Legal test was positive. Analytical data for this and other products are given in Table I.

Later fractions from the chromatogram contained more nitrogen, gave dubious Legal tests, and probably were the result of partial reaction with a second equivalent of amine.

With n-butylamine (III, IV). This was obtained in almost quantitative yield by the same procedure. After recrystallization from benzene-petroleum ether, a monohydrate which softened at 90° and decomposed at 105–107° was obtained. When dried at 100° over phosphorus pentoxide *in vacuo* the compound melted to a glass which gave satisfactory analytical figures for the anhydrous lactam.

With tryptamine (V). Equimolar portions of strophanthidin (404 mg.) and tryptamine (160 mg.) were intimately mixed and dissolved in 15 ml. of absolute ethanol. The solution was refluxed in the steam bath for 2 hr., after which half the volume of solvent was removed by distillation. Crystallization occurred in the hot solution and, after cooling, the white material was collected, washed twice with absolute ethanol, and air dried. It gave a positive Legal test and melted with decomposition at 200–220°. On treatment with 3% hydrochloric acid, it partially dissolved. After filtering, the filtrate was neutralized with sodium carbonate solution and the precipitate was collected, dried, and taken up in boiling absolute ethanol. Pentane was added to the filtered alcohol solution to the appearance of turbidity. On refrigeration white rosettes of needles, m.p. 217–220°, appeared.

In a subsequent preparation, the product crystallized directly from the alcoholic reaction mixture without treatment with hydrochloric acid. A second crop was obtained by addition of pentane to the mother liquor. Elimination of the hydrochloric acid treatment is desirable as some decomposition of the product attends its use.

With 1-diethylamino-4-aminopentane (VI). *Procedure A.* To a solution of 1 g. of strophanthidin in 7 ml. of 1-diethylamino-4-aminopentane purified over the dithiocarbamate[14] a mixture of 25 ml. of benzene and 25 ml. of toluene was added and the clear solution was refluxed overnight. After removal of most of the solvent at the water pump, addition of petroleum ether precipitated the product as an oily mass. After decanting the solvents, the oily residue was taken up in dilute hydrochloric acid, filtered from insoluble material, and the lactam was precipitated as an oily mass by addition of potassium carbonate solution. This was lifted from the liquid and, after air drying, the substance solidified. It was further purified by precipitation from benzene by petroleum ether, a second precipitation from dilute hydrochloric acid by potassium carbonate, and final crystallization from benzene-petroleum ether. The yield of material, m.p. 92–95° dec. after softening at 80°, was 100 mg. It gave a positive Legal test. For analysis it was dried *in vacuo* at 56°.

Procedure B. To a solution of 1 g. of strophanthidin in 10 ml. of hot cyclohexanone was added 3 ml. of 1-diethylamino-4-aminopentane.[14] After heating the mixture at 140–150° in an oil bath for 15 min. and cooling, the product was precipitated by addition of petroleum ether. After purification by the hydrochloric acid procedure described above, 500 mg. of product was obtained.

With glycine ethyl ester (VII). A mixture of 0.44 g. of freshly prepared glycine ethyl ester and 2 g. of strophanthidin was warmed on the steam bath in a flask provided with a calcium chloride tube for 20 min. About half of the strophanthidin went into solution and the reaction product appeared as a thick viscous liquid mixed with unchanged strophanthidin. The mixture was extracted twice with ether to remove unchanged glycine ester and then with cold dilute hydrochloric acid to remove the product from unchanged strophanthidin. After precipitation of the product by slowly adding solid potassium carbonate to the acid extract, the oily material was removed and air dried, on which it solidified. It was taken up in boiling benzene and filtered. On cooling and addition of petroleum ether an oil separated. The benzene-petroleum ether purification was repeated three times and finally a crystalline product was obtained. The yield was 1 g. The substance gave a positive Legal test, softened at 100°, and melted with decomposition at 115–119°. For analysis it was dried *in vacuo* at 65°.

With dl-alanine ethyl ester (VIII). The procedure was the same as that used with glycine ethyl ester. The product, obtained in 55% yield with complete recovery of starting material, formed a crystalline mass, m.p. 118–122° dec. with softening at 100°. For analysis it was dried *in vacuo* at 65°.

With dl-tryptophan methyl ester (IX). This was prepared in the same manner as was the product from glycine ethyl ester except that a 30-min. heating period gave a product of highest purity in maximum yield. The yield was 52% with complete recovery of starting materials. The substance decomposed at 155–160° with softening at 120°. For analysis it was dried *in vacuo* at 80°.

(14) R. G. Jones, *Ind. Eng. Chem., Anal. Ed.*, **16**, 431 (1944).

With piperidine (XXV). *Procedure A.* To a solution of 1 g. of strophanthidin in 10 ml. of warm cyclohexanone was added 2 ml. of dry and freshly distilled piperidine. The mixture was heated at 150° in an oil bath for 15 min. After cooling, addition of three volumes of pentane precipitated a semisolid mass. After chilling for 2 hr., the supernatant liquid was decanted and the residual oil was extracted with 3% hydrochloric acid which left 577 mg. of crude insoluble unreacted strophanthidin. The product was precipitated from the acid solution by addition of dilute sodium carbonate solution. Precipitation from benzene by pentane gave an amorphous off-white powder which was chromatographed in benzene solution over alumina. Elution with benzene-methanol gave 110 mg. of crystalline material, m.p. 195–197°, from 350 mg. of crude product. The Legal test was negative. This is the product of the reaction of strophanthidin with two equivalents of piperidine with elimination of one water. This material contains methanol of crystallization. Solvent free material can be obtained from benzene as described below. For analysis it was dried *in vacuo* at 100°, which gave a solvent free product.

Anal. Calcd. for $C_{33}H_{52}N_2O_5$: C, 71.1; H, 9.4; N, 5.0. Found: C, 70.9; H, 9.4; N, 5.1.

The later fractions from the chromatogram gave material which displayed a positive Legal test and which gave analytical figures for nitrogen corresponding to reaction of strophanthidin with one equivalent of piperidine. However, the carbon and hydrogen figures did not agree with this interpretation. The compound is under further investigation.

Procedure B. A solution of 1 g. of strophanthidin in 10 ml. of cyclohexanone was diluted with 50 ml. of a 1:1 mixture of benzene and toluene. After refluxing for 48 hr. on the steam bath, the solvents were removed at the water pump and the residue was worked up as in Procedure A without chromatography. The product formed white prisms after several crystallizations from benzene-petroleum ether and finally from benzene. It contracts at 170°, begins to darken at 250°, and decomposes at 255°. The yield was 800 mg.

Anal. Found: C, 70.8; H, 9.6; N, 5.0.

With morpholine. The product of the reaction of strophanthidin with two equivalents of morpholine was obtained in almost quantitative yield by Procedure A as given for the piperidine compound. It softened at 158–160° and melted with decomposition at 180°. The Legal test was negative.

Anal. Calcd. for $C_{31}H_{48}N_2O_7$: C, 66.4; H, 8.6; N, 4.9. Found: C, 66.1; H, 8.5; N, 4.6.

Reaction of strophanthidin benzoate with n-propylamine. A solution of 1 g. of strophanthidin benzoate in 5 ml. of *n*-propylamine was warmed in an open flask on the steam bath for 10 min., during which the amine evaporated leaving a brown gum. Last traces of the amine were removed at the water pump and the residue was extracted with dilute hydrochloric acid. About one-fourth of the residue went into solution leaving unchanged strophanthidin benzoate. The acid extract was made alkaline with potassium carbonate solution and the precipitate was recrystallized from benzene-petroleum ether to give 0.3 g. of material which softened at 100°, melted at 118–120°, resolidified, and finally melted with decomposition at 195°. The Legal test was positive.

Anal. Calcd. for $C_{33}H_{43}NO_6$: C, 72.1; H, 7.8; N, 2.6. Found: C, 71.9; H, 7.9; N, 2.8.

Reaction of strophanthidin oxime with n-propylamine. A solution of 1 g. of strophanthidin oxime in 10 ml. of *n*-propylamine was warmed under an air condenser on the steam bath for 30 min. The condenser was removed and the amine was evaporated completely. Extraction of the residue with dilute hydrochloric acid left 0.5 g. of insoluble unchanged oxime. The acid filtrate was made alkaline with potassium carbonate solution and the white precipitate was collected. As this did not give a positive Legal test, it was put aside for future investigation. The alkaline filtrate was almost saturated with solid potassium carbonate with cooling and the solid which was salted out was collected, dried, and extracted with boiling benzene to remove traces of the material giving the negative Legal test. It was finally extracted with boiling chloroform, the chloroform solution was filtered from inorganic salts, and pentane was added. The oxime which separated as white crystals underwent a transition at 130–140° and the new solid melted at 200° with decomposition. The yield was 0.1 g. and the Legal test was positive.

Anal. Calcd. for $C_{26}H_{40}N_2O_5$: C, 67.8; H, 8.7; N, 6.0. Found: C, 67.7; H, 8.7; N, 5.6.

Reaction of β-phenyl-$\Delta^{\alpha,\beta}$-butenolide with n-propylamine (XXI). A solution of 1 g. of the butenolide and 5 ml. of *n*-propylamine in 50 ml. of dry benzene was refluxed on the steam bath for 12 hr. and the benzene was removed under reduced pressure. The oily residue was taken up in 5% hydrochloric acid leaving 0.2 g. of undissolved material. The insoluble material gave a positive Legal test which, in contrast to the negative test displayed by the acid insoluble fraction, was obtained when the butenoide was heated directly with propylamine without the use of benzene as solvent. In the latter instance a portion of the butenolide may have been altered in a different manner. The acid solution was repeatedly extracted with ether to remove non-nitrogenous material and made alkaline with ammonia. The alkaline solution was extracted with ether and the alkaline solution put aside. The combined ether extracts were dried over anhydrous sodium sulfate. Removal of the solvent left 0.6 g. of a viscous transparent liquid which did not crystallize and gave a negative Legal test. The oil was taken up in 15 ml. of absolute ether and a dilute ethereal solution of bromine was added until the color of bromine persisted. Reaction with bromine was instantaneous. Removal of the ether under reduced pressure left a viscous residue which was taken up in 3 ml. of benzene. Dry ether was added until precipitation was complete. The supernatant liquid was decanted and the precipitation repeated twice. On stirring, the sticky residue crystallized. Further recrystallization from benzene-ether and finally from benzene gave 0.3 g. of white crystals, m.p. 138°. For analysis it was dried at 78° *in vacuo*.

Anal. Calcd. for $C_{16}H_{25}BrN_2O$: C, 56.3; H, 7.3; N, 8.2; Br, 23.5. Found: C, 56.5; H, 7.6; N, 7.8; Br, 23.6.

The alkaline water layer from the above ether extraction was taken to dryness and the residue was extracted with chloroform. From the chloroform extracts 0.1 g. of material, m.p. 160°, which gave a positive Legal test was obtained. On attempted purification the substance was changed to one with which the Legal test was negative and which was not investigated further.

Reaction of β-phenyl-$\Delta^{\alpha,\beta}$-butenolide with morpholine. (XXVIII, R = $-CH_2CH_2OCH_2CH_2-$) The procedure was the same as in the reaction of the butenolide with *n*-propylamine. The residue from removal of the benzene was completely soluble in dilute hydrochloric acid. The solid residue was extracted several times with warm acetone leaving 0.5 g. of acetone insoluble material, m.p. 105–106°. Attempted purification resulted in a deep-seated change. The substance was therefore converted to the oxime which formed white prisms, m.p. 140–141°, from ethanol. Analyses corresponded to the oxime of β-phenyl-β-formylpropionylmorpholine.

Anal. Calcd. for $C_{14}H_{17}N_2O_3$: C, 64.4; H, 6.5; N, 10.7. Found: C, 64.4; H, 6.8; N, 10.4.

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