hydrous conditions with a gradual increase of temperature to 80° during the next 2 hr. The reaction mixture was kept at 80° for 3 hr. The product which precipitated upon cooling was isolated by filtration and recrystallized from petroleum ether (bp 90–120°) to give the lactone in colorless flakes (yield 8 g), mp 93–94°.[4,5] The molecular weight was determined in chloroform and found to be 158. The infrared spectrum exhibited the lactone carbonyl absorption at 1802 and 1790 cm^{-1}.[7,12]

Anal. Calcd for $C_{10}H_8O_2$: C, 75.0; H, 5.03. Found: C, 74.9; H, 4.95.

α-(2-Hydroxy-3,5-dibromobenzylidene)-γ-phenyl-$\Delta^{\beta,\gamma}$-butenolide (Ia).—Following cooling of the 10-ml chloroform solution of γ-phenyl-$\Delta^{\beta,\gamma}$-butenolide (1.6 g) and 2-hydroxy-3,5-dibromobenzaldehyde (2.8 g), 6 drops of dried and freshly redistilled triethylamine was added over the next 30 min while the reaction vessel was continuously swirled in ice water. After 5 hr of storage at 4° the crystalline material consisting of Ia and IIa was collected by filtration. The precipitate (1.22 g), washed with 5 ml of cold ethanol, was subsequently extracted for 5 min at room temperature with 5 ml of chloroform. After removal of the insoluble coumarin IIa the filtrate was stored at 4° with exclusion of light. The resulting yellow needles were collected the following day, washed with 1 ml of ethanol, and dried *in vacuo* at room temperature again with exclusion of light, yielding 0.4 g, mp 200–205°. The infrared spectrum showed the phenol absorption at 3370 and the lactone carbonyl absorption at 1780 and 1768 cm^{-1} (poorly resolved doublet). The spectrum between 200 and 600 mμ exhibited four maxima: 231 mμ (ε 15,300), 250 (16,300), 404 (21,400), and 505 (3200).

Anal. Calcd for $C_{17}H_{10}Br_2O_3$: C, 48.4; H, 2.39; Br, 37.9. Found: C, 48.6; H, 2.47; Br, 37.9.

α-(2-Hydroxy-3,5-dibromobenzylidene)-γ-(4-methoxyphenyl)-$\Delta^{\beta,\gamma}$-butenolide (Ib).—As in the condensation reaction above γ-(4-methoxyphenyl)-$\Delta^{\beta,\gamma}$-butenolide[5-7] (1.9 g) was allowed to react with 2-hydroxy-3,5-dibromobenzaldehyde in the presence of 6 drops of triethylamine at 0°. The resulting crystalline mixture was extracted at room temperature with 25 ml of chloroform for 15 min and undissolved coumarin IIb was removed by filtration. The filtrate, stored overnight at 4°, yielded orange needles which were once more recrystallized from chloroform in the manner just described yielding 1.2 g, mp 205–207°. The infrared spectrum exhibited the phenol absorption at 3380 and the carbonyl absorption at 1780 with a shoulder at 1770 cm^{-1}. In the ultraviolet and visible region the following maxima were observed: 225 mμ (ε 12,100), 270 (13,700), 416 (23,800), and 500 (2900).

Anal. Calcd for $C_{18}H_{12}Br_2O_4$: C, 47.8; H, 2.68; Br, 35.4. Found: C, 47.9; H, 2.80; Br, 35.4.

3-Phenacyl-6,8-dibromocoumarin (IIa). Method A.—The γ-phenyl-$\Delta^{\beta,\gamma}$-butenolide (0.8 g) and 2-hydroxy-3,5-dibromobenzaldehyde (1.4 g) were dissolved in 10 ml of chloroform and refluxed for 1 hr with 0.5 ml of pyridine (or triethylamine). An infrared spectrum of the crude reaction product revealed the absence of Ia. Upon removal of the solvent a residue resulted which was recrystallized from a large excess of ethanol, yielding 1.6 g, mp 235–237°.

The infrared spectrum showed the δ-lactone carbonyl absorption at 1730 and the ketone carbonyl absorption at 1665 cm^{-1}. A weak but broad absorption in the hydroxyl region centered around 3440 cm^{-1} suggested that the phenacyl group in position 3 of the coumarin is partially enolized. In the ultraviolet spectrum three maxima were recorded: 228 mμ (ε 29,100), 280 (17,200), and 325 (4900).

Anal. Calcd for $C_{17}H_{10}Br_2O_3$: C, 48.4; H, 2.39; Br, 37.9. Found: C, 48.5; H, 2.54; Br, 37.9.

Method B.—Two-tenths of a gram of Ia was dissolved in 2 ml of pyridine and heated for 1 hr to 80°. The solvent was removed and the remaining residue was recrystallized from ethanol, yielding 180 mg, mp 237°. Mixture melting points with samples prepared according to method A were not depressed.

3-(4-Methoxyphenacyl)-6,8-dibromocoumarin (IIb). Method A.—A solution of γ-(4-methoxyphenyl)-$\Delta^{\beta,\gamma}$-butenolide (1.3 g) and 2-hydroxy-3,5-dibromobenzaldehyde (1.8 g) in 10 ml of chloroform was refluxed with 0.5 ml of pyridine (or triethylamine). The solvent was removed after 1 hr *in vacuo* and the resulting solid material was crystallized from chloroform in white needles, yielding 2.2 g, mp 225–226°.

The lactone and the ketone carbonyl absorptions were located at 1730 and 1665 cm^{-1}, respectively. A broad absorption of considerable intensity centered around 3440 cm^{-1} suggests an increased enolization of IIb as compared with IIa. The ultraviolet spectrum showed three maxima at 220 mμ (ε 35,700), 284 (32,900), and 325 (5800).

Anal. Calcd for $C_{18}H_{12}Br_2O_4$: C, 47.8; H, 2.68; Br, 35.4. Found: C, 47.5; H, 2.63; Br, 35.7.

Method B.—Two-tenths of a gram of butenolide Ib was converted to coumarin IIb as described for IIa (method B). The product was recrystallized from boiling chloroform yielding white needles, (190 mg), mp 225–226°. Mixture melting points with samples from preparation A remained unchanged.

3-Phenacylcoumarin.—A solution of γ-phenyl-$\Delta^{\beta,\gamma}$-butenolide (0.8 g) and 2-hydroxybenzaldehyde (0.6 g) was refluxed for 1 hr in 10 ml of chloroform in the presence of 0.5 ml of triethylamine. After this period the volatile substances were removed under reduced pressure. From the resulting solid material a red by-product was extracted with 10 ml of warm methanol. The product was subsequently recrystallized from chloroform–benzene (1:1) and finally, to remove the last traces of color, from 500 ml of ether, yield 0.9 g, mp 165–166°.[1] Characteristic absorption bands in the infrared spectrum were located at 3450 (broad), 1720, and 1685 cm^{-1}. In the ultraviolet region three maxima were detected: 242 mμ (ε 14,400), 276 (15,000), and 310 (8200).

Anal. Calcd for $C_{17}H_{12}O_3$: C, 77.3; H, 4.57. Found: C, 77.5; H, 4.26.

Registry No.—α-Phenyl-$\Delta^{\beta,\gamma}$-butenolide, 1955-39-1; Ia, 10075-43-7; Ib, 10075-44-2; IIa, 10075-45-3; IIb, 10075-46-4; 3-phenacylcoumarin, 10075-47-5.

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Oxidation of Steroid Digitonides[1]

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Of the many ways known for the protection of hydroxyl groups,[2] one of the most unique is the use of digitonin for the protection of a steroidal 3β-hydroxyl group.[3,4] A remarkable feature of this method is that the digitonin is not attached to the steroid molecule by a covalent bond; rather, digitonides are molecular complexes, easily disrupted by dissolving in pyridine.[5] Steroidal hydroxyl groups at C-17[3] and at C-20[4] have been oxidized with simultaneous digitonide protection of the 3β-hydroxyl group, but oxidations by this method at positions closer to C-3 have not been reported. We have explored the possibility of carrying out selective oxidations at C-16, C-12, C-11, C-6, and C-2 on steroid digitonides.

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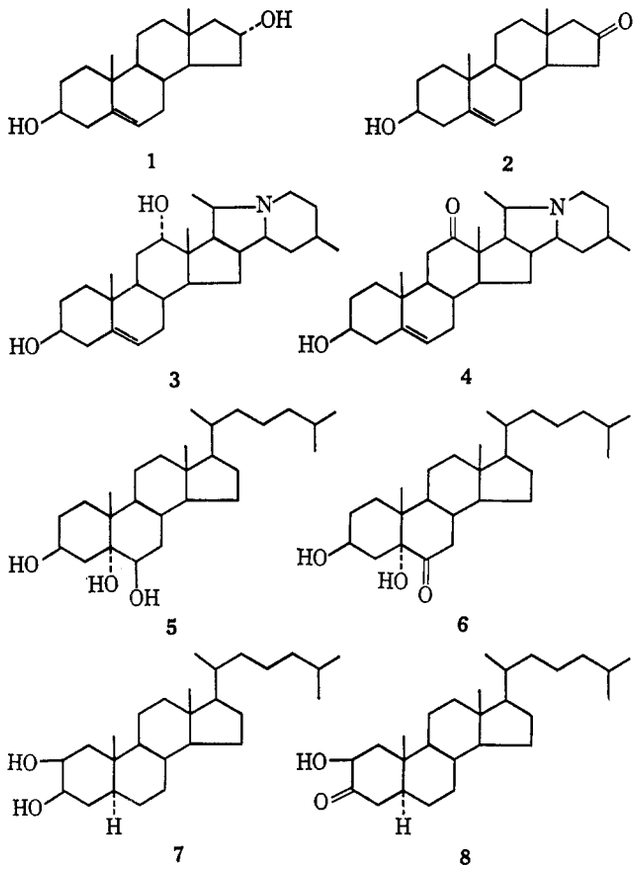
CHART I



5-Androstene-3β,16α-diol (**1**) (Chart I) was oxidized to 5-androsten-3β-ol-16-one (**2**) in a manner identical with the oxidation of 5α-androstane-3β,17β-diol.[3] Rubijervine (**3**) was oxidized to 12-rubijervone (**4**) by a modification of the original method (see Experimental Section), but similar oxidative attempts with methyl 3β,12α-dihydroxycholanate,[6] 3β,12α-dihydroxycholanic acid,[7] and 5α-pregna-16-ene-3β,12β-diol-20-one[8] failed. 5-Pregnene-3β,11α-diol-20-one[9] does not form an insoluble digitonide. Cholestane-2β,5α,6β-triol (**5**) gave cholestane-3β,5α-diol-6-one (**6**), though in only 27% yield.[10] Oxidation of cholestane-2β,3β-diol (**7**) gave mostly the seco diacid plus a small yield of an hydroxy ketone tentatively identified as cholestan-2β-ol-3-one (**8**),[11b] implying digitonin protection of the 2-hydroxyl rather than the 3-hydroxyl group. Though this was not predicted, it may be pointed out that both 2β-cholestanol[12] and cholestan-2β-ol-3-one form digitonides.

On the basis of other reports[3,4] and this somewhat limited series, it seems clear that the Kupfer, Forchielli, and Dorfman procedure works best for steroids in which the hydroxyl group undergoing oxidation is at least as far away from C-3 as the D ring. Apparently the presence of an hydroxyl group in the C ring often prevents digitonide formation or else gives rise to digitonides which do not survive the oxidation step.

Experimental Section[13]

Oxidation of 5-Androstene-3β,16α-diol (1).—A hot solution of 375 mg of digitonin in 18 ml of 90% ethanol was added to a solution of 75 mg of 5-androstene-3β,16α-diol in 18 ml of 90% ethanol. The precipitate that formed on cooling was collected by centrifugation, washed with 90% ethanol and ether, and then dried, yield 320 mg.

The digitonide was dissolved in 15 ml of acetic acid. A solution of 180 mg of CrO_3 in 6 ml of H_2O and 9 ml of acetic acid was added; after 30 min at room temperature the excess CrO_3 was reduced with a few drops of a saturated aqueous solution of Na_2SO_3. The green solution was diluted with 375 ml of H_2O, refrigerated, and centrifuged; the precipitate was washed with H_2O and dried in a vacuum desiccator over P_2O_5, yield 134 mg.

The digitonide from the oxidation step was dissolved in a minimum of pyridine (about 1 ml) and kept at room temperature for 7 hr; the solution was diluted with 375 ml of ether and centrifuged. The ethereal solution was washed with dilute H_2SO_4, dried over anhydrous Na_2SO_4, and evaporated, yield 42 mg (56%), λ_{max} 5.72 μ (C=O). After recrystallization in acetone–H_2O the product **2** melted at 161–162°; recrystallization in methanol raised the melting point to 162–163° (lit. mp 163.5–165.0,[14] and 168–169°[15]).

Oxidation of Rubijervine (3).—The digitonide (2.80 g) was prepared similarly to that of **1** from 815 mg of rubijervine. Its solution in 160 ml of AcOH was treated with a solution of 1.92 g of CrO_3 in 64 ml of H_2O and 96 ml of AcOH. After 30 min the solution was made green with aqueous Na_2SO_3 and diluted with 2 l. of H_2O; the solution remained clear.[16] It was made basic by cautious addition of solid $NaHCO_3$ and extracted with four 450-ml portions of ether; the portions of thick emulsion that survived centrifugation were combined and vacuum dried. The total ether extract was washed with 200 ml of H_2O, dried over Na_2SO_4, and evaporated, yield 276 mg of solid. Chromatography on 14 g of Al_2O_3 gave small fractions eluted by benzene and ether, then 139 mg of 12-rubijervone (**4**) eluted by 5% methanol in ether; recrystallized in acetone it melted at 228–230° dec (lit.[17] mp 229.5–232°), λ_{max} 3.05 (OH) and 5.86 μ (C=O). Extraction of the dried emulsion with ether gave 17 mg and with acetone an additional 21 mg of product with infrared spectra identical with that of the main chromatographic fraction; total yield of **4** was 177 mg, 22%.

Oxidation of Cholestane-3β,5α,6β-triol (5).—Precipitation with digitonin and oxidation with CrO_3 of 30 mg of **5** in a manner similar to that described for **1** gave 8 mg of crude product (**6**), which on recrystallization in methanol melted at 218–221°. This was difficult to obtain in high-melting form, but combined product from several similar runs, decolorized and recrystallized from methanol, exhibited an infrared curve very similar to that of authentic **6**. Cholestane-3β,5α-diol-6-one, from hydrolysis of the 3-acetate,[18] melted at 230–241° (lit.[19] mp 237°). Cholestan-5α-ol-3,6-dione[18] melted at 247–252° (lit. mp 248–251°).

(6) Prepared by formolysis of methyl desoxycholate 3-monotosylate: F. C. Chang and N. F. Wood, *J. Org. Chem.*, **30**, 1718 (1965). By our modified method only methyl 3,12-diketocholanate was obtained from the oxidation studies.

(7) Recovery of digitonide after the oxidation was only 1.5%.

(8) From hydrolysis of the diacetate (Mann Research Laboratories, No. 6505). No digitonide precipitated after the oxidation.

(9) Prepared by isomerization of 3,5α-cyclopregnane-6β,11α-diol-20-one, generously supplied by the Upjohn Co. The use of 0.1 *N* HCl in refluxing acetone [W. J. Wechter and H. C. Murray, *J. Org. Chem.*, **28**, 755 (1963)] in our hands usually gave predominately 3β-chloro-5-pregnen-11α-ol-20-one. Isomerization was effected in good yield using 0.2 *N* H_2SO_4 in acetone at room temperature overnight.

(10) While it is true that this triol can be oxidized to the 3,5-diol-6-one (**6**) in higher yield in the absence of digitonin,[11a] the significance of our experiment is that *under these oxidation conditions* the 3,6-dione is obtained when digitonin is absent.

(11) (a) B. Ellis and V. Petrow, *J. Chem. Soc.*, 1078 (1939). (b) The possibility that some 3β-ol-2-one was present in the crude product, but not isolated, is not ruled out.

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(13) Melting points were taken on an electrical hot stage or a Unimelt apparatus and are uncorrected. Infrared spectra were determined as mineral oil mulls with an Infracord. Ultraviolet spectra were obtained on methanolic solutions using a Cary spectrophotometer. Optical rotations were determined in 2% chloroform solutions with a Keston polarimeter attachment to a Beckman DU spectrophotometer. Microanalyses were by Galbraith Laboratories, Knoxville, Tenn.

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(15) J. Fajkos and F. Šorm, *Chem. Listy*, **47**, 1207 (1953).

(16) In an earlier small-scale experiment, dilution with the usual 12.5 volumes of H_2O also gave a clear solution.

(17) S. W. Pelletier and D. M. Locke, *J. Am. Chem. Soc.*, **79**, 4531 (1957).

(18) R. E. Marker and E. Rohrmann, *ibid.*, **62**, 516 (1940).

(19) B. Ellis and V. Petrow, *J. Chem. Soc.*, 1078 (1939).

Oxidation of Cholestane-2β,3β-diol (7).—Digitonin precipitation and CrO_3 oxidation of 91 mg of cholestane-2β,3β-diol[20] gave 35 mg of crude product. Thin layer chromatography on silica gel gave a band, R_f 0.25–0.35, when developed in 4% methanol in benzene; elution gave a product with OH and C=O bands in the infrared region but which could not be crystallized.

The reaction was repeated with 450 mg of 2,3-diol using a 9-min reaction time; after centrifugation to recover digitonide of the oxidized product, the supernatant was extracted with ether. Evaporation under vacuum gave 173 mg of a solid which, after recrystallization in benzene, exhibited melting point, mixture melting point, and infrared spectra identical with those 2,3-secocholestane-2,3-dioic acid,[21] mp 200–202° (lit.[22] mp 196–197°). The usual work-up of the digitonide gave 72 mg of product, which was chromatographed on 5 g of Florisil. The major cut, 45 mg eluted by 10% methanol in benzene, was chromatographed on a thin layer silica gel plate and developed in 10% methanol in benzene. Two bands were observed by aqueous $KMnO_4$ spraying, R_f 0.33 and 0.70. The latter was eluted giving 21 mg which on recrystallization in 95% ethanol melted at 109.8–122.4° and gave an infrared curve very similar to that of authentic **8**; our product when mixed with cholestan-2β-ol-3-one melted at 119–124°; when mixed with cholestan-3β-ol-2-one it melted at 110–130°. Cholestan-2β-ol-3-one acetate,[20] mp 143.2–147.0° (lit. mp 145.3–146.3°), was hydrolyzed with aqueous K_2CO_3 according to Williamson and Johnson[20] to give the 2β-ol-3-one (**8**), mp 108–122°. Several recrystallizations in acetone–water raised the melting point to 118–124°, $[\alpha]D$ +48°, λ_{max} 2.85 (OH) and 5.85 μ (C=O). One further recrystallization gave the analytical sample, mp 120.2–123.0°; it is precipitated by digitonin.

Anal. Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.45; H, 11.23.

Cholestan-3β-ol-2-one acetate,[20] mp 143.2–146.2° (lit.[20] mp 145.5–146.1°), melted at 125–145° when mixed with the 2β-ol-3-one acetate. It was hydrolyzed in alcoholic KOH to give cholestan-3β-ol-2-one, mp 117–132° (lit.[23] mp 104–105), which when mixed with **8** melted at 108–124°.

Registry No.—**1**, 3642-89-5; **3**, 10146-87-9; **5**, 1253-84-5; **7**, 10146-89-1; digitonin, 35-62-1.

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Amidinourea Formate, a Precursor of 2-Amino-4-hydroxy-*s*-triazine[1a]

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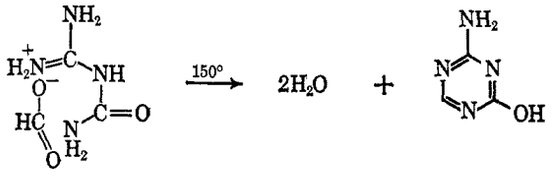
Grundmann, *et al.*,[2] and Piskala and Gut[3] described a reaction between formic acid and cyanoguanidine which led to the production of 2-amino-4-hydroxy-*s*-triazine (II). Inasmuch as several *s*-triazines had been found to inhibit purine-utilizing enzymes[4–6] we repeated their procedure and found that an intermediate compound in their synthesis, which they took to be a hydrate of 2-amino-4-hydroxy-*s*-triazine, was in fact guanylurea formate (I). The identification of this intermediate and the implications of its conversion to 2-amino-4-hydroxy-*s*-triazine form the body of this report.



I II

The profound differences in solubility, melting point, and ultraviolet spectra observed between the intermediate (I) and the *s*-triazine (II),[7] together with our finding that the intermediate (I) was a competitive inhibitor of adenine aminohydrolase[8] whereas the triazine (II) was not suggested that the intermediate was something other than a hydrate of the *s*-triazine. Karl Fischer titration[9] of the intermediate confirmed this suspicion, indicating a water content of not more than 0.25%.

Titration of an aqueous solution of the intermediate in the pH range 5 to 12 indicated a pK_a of 8.0 and an equivalent weight of 149 ± 3, while a mass spectral analysis[10] indicated a parent ion of *m/e* 148. The pK_a of 8.0 suggested that one must expect to isolate the intermediate from an acidic medium as a salt. The conductivity of aqueous solutions of the intermediate indicated that it was a salt. Subsequently, 0.250 mequiv of the intermediate was found to displace 0.245 mequiv of chloride from a column of an anion-exchange resin in the chloride form.[7] The chloride salt eluted from this column was chromatographically identical[7] with amidinourea chloride prepared from commercial amidinourea sulfate.

The intermediate was therefore presumed to be amidinourea formate. This identification was confirmed by several lines of evidence. Amidinourea formate was prepared from commercial amidinourea sulfate and was found to be identical with the intermediate by several criteria. Thus, they exhibited identical ultraviolet and infrared absorption spectra. Both substances were competitive inhibitors of adenine aminohydrolase and at pH 7.0 and 25° exhibited the same inhibition constant which was 0.002 *M*.[8] The pK_a of amidinourea is known to be 8.0.[11] Finally, the elemental analysis of the intermediate was in accord with that expected of amidinourea formate.

The data in this report establish that the first stable product of the reaction of cyanoguanidine with dry formic acid is amidinourea formate. The reaction therefore involves the hydrolysis of the nitrile group, a

(1) (a) Supported in full by Grant No. GM-10287-04 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.; (b) Special Postdoctoral Fellow of the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.; (c) Research Career Development Awardee of the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
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