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.

(20) K. L. Williamson and W. S. Johnson, *J. Org. Chem.*, **26**, 4563 (1961).
(21) R. E. Marker and L. Plambeck, Jr., *J. Am. Chem. Soc.*, **61**, 1332 (1939).
(22) B. Heath-Brown, I. M. Heilbron, and E. R. H. Jones, *J. Chem. Soc.*, 1482 (1940).
(23) L. Ruzicka, P. A. Plattner, and M. Furrer, *Helv. Chim. Acta*, **27**, 727 (1944).

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

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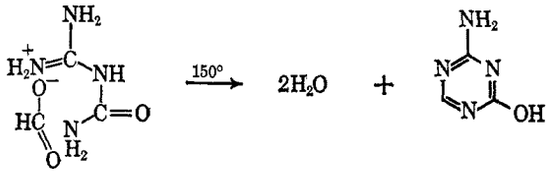
Grundmann, *et al.*,[2] and Piskala and Gut[3] described a reaction between formic acid and cyanoguanidine which led to the production of 2-amino-4-hydroxy-*s*-triazine (II). Inasmuch as several *s*-triazines had been found to inhibit purine-utilizing enzymes[4–6] we repeated their procedure and found 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

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(2) C. Grundmann, L. Schwennicke, and E. Beyer, *Chem. Ber.*, **87**, 19 (1954); Deutsche Hydrierwerke. A. G., German Patent 861 384 (1941); C. Grundmann, *Chem. Zent.* **124**, 3152 (1953).
(3) A. Piskala and J. Gut, *Collection Czech. Chem. Commun.*, **28**, 1681 (1963).
(4) I. Fridovich, *Federation Proc.*, **24**, 594 (1965).
(5) I. Fridovich, *Biochemistry*, **4**, 1098 (1965).
(6) I. Fridovich, *J. Biol. Chem.*, **240**, 2491 (1965).
(7) See the Experimental Section.
(8) R. Hartenstein and I. Fridovich, *J. Biol. Chem.*, **242**, 740 (1967).
(9) J. Mitchell and J. M. Smith, "Aquametry," Interscience Publishing Co., New York, N. Y., 1948.
(10) Mass spectral analyses were kindly performed by Dr. J. Ruth of the Liggett and Myers Tobacco Corp.
(11) R. S. Hirt and R. G. Schmitt, *Spectrochim. Acta*, **12**, 127 (1958).

reaction reported to be acid catalyzed for acylated cyanoguanidines.[12] The water necessary for this hydrolysis must have been provided by the decomposition of the initially dry formic acid to CO plus H_2O. Vigorous evolution of a gas, presumably CO, was observed during the reaction, at temperatures well below the boiling point of formic acid. Since formic acid, *per se*, is stable under these conditions, one must conclude that cyanoguanidine somehow catalyzes the decomposition of formic acid. Its ability to do so, albeit at a reduced rate, was demonstrated even at room temperature.

The cyclization of guanylurea formate by heating at 150° represents a novel method for synthesizing the *s*-triazine ring. Since this reaction results in the incorporation of the carbon of formate into the *s*-triazine ring, it may provide the basis for the synthesis of several disubstituted *s*-triazines from a variety of formate salts of acylcyanoguanidines[12,13] or from other carboxylic acid salts of amidinourea.

Experimental Section

Melting points were measured on a Fisher-Johns apparatus. Ultraviolet absorption spectra were recorded with a Cary Model 15 spectrophotometer. Infrared spectra were obtained from pressed KBr disks with a Perkin-Elmer Model 337 spectrophotometer. Mass spectra were obtained on a Bendix time of flight spectrometer at a probe temperature of 105°. Paper chromatography was performed on Whatman 3 MM with *n*-butyl alcohol–methyl alcohol–water (4:1:1) as the solvent. Spots on the paper chromatograms were visualized with a spray active N–H group.[14] Elemental analyses were performed by the Galbraith Laboratories of Knoxville, Tenn.

Amidinourea Formate.—Cyanoguanidine (8.6 g) and 13.8 g of dry formic acid were heated under reflux to 80°. Heating was discontinued and the temperature of the reaction mixture was allowed to rise to 120°. An ice bath was kept at hand to control excessive rates of reaction. After 10 min the reaction subsided and the reaction mixture solidified. The solid was washed with alcohol and recrystallized twice from water: mp 140–145° (with effervescence); $\lambda_{max}^{0.1\ N\ NaOH}$ 219 mμ (ε 21,000); J = 1.5 ($HCOO^-$) and 2.0–3.0 cps (7 H) in dimethyl sulfoxide; J = 0.6 ($HCOO^-$), 1.8 (1 H), and 2.5 cps (6 H) in trifluoroacetic acid; $\bar{\nu}_{max}$ 3440, 3300, 3100 (NH, $CONH_2$), 2800, 2720 (acidic H), 1755, 1715, 1645, 1610, 1470 (COO^-, $CONH_2$, NH), 1395 (NH), 1365 (C=O), 1090, 918, 855, 770, 730, 710 cm^{-1} (NH, C=O, CN); solubility (at 25°) 0.3 g/100 ml of H_2O, 4.2 g/100 ml of DMSO. *Anal.* Calcd for $C_3H_8N_4O_3$: C, 24.32; H, 5.40; N, 37.82. Found: C, 24.43, 25.24; H, 5.40, 5.74; N, 38.45, 37.48.

Conversion of Amidinourea Sulfate to Amidinourea Formate.—The sulfate (4.0 g) was heated into solution with 20 ml water and allowed to cool to room temperature; 4.16 g of $Ba(OH)_2 \cdot 8H_2O$ was added, the precipitate was removed, and 2.0 ml of dry formic acid was added. The mixture was dried *in vacuo* at 40°. The melting point, ultraviolet, infrared, and chromatographic characteristics were identical with those given above.

Amidinourea Hydrochloride.—Amidinourea formate (740 mg, intermediate) in 200 ml of distilled water was passed through a 1 × 20 cm column of Bio-Rad AG 21K resin in the chloride form. The effluent was lyophilized and a sample was submitted without further treatment for analysis, mp 140–142° dec. *Anal.* Calcd for $C_2H_7ClN_4O$: C, 17.44; H, 5.07; Cl, 25.28; N, 40.60. Found: C, 17.86, 17.84; H, 4.99, 5.10; Cl, 24.70, 24.53; N, 40.16, 40.26.

2-Amino-4-hydroxy-*s*-triazine.—Amidinourea formate (1 g) was placed as a thin layer in a 10-cm petri dish into a preheated oven at 150° for 90 min, yielding 671 mg (81.5%): mp >300°; $\lambda_{max}^{0.01\ N\ NaOH}$ 250 mμ (ε 3300); solubility (at 25°) 0.0015 g/100 ml of H_2O.

Registry No.—I, 10043-39-7; II, 4040-10-2; amidinourea hydrochloride, 926-72-7.

(12) P. Adams, D. W. Kaiser, D. E. Nagy, G. A. Peters, R. L. Sperry, and J. T. Thurston, *J. Org. Chem.*, **17**, 1162 (1952).
(13) D. W. Kaiser and J. T. Thurston, *Chem. Abstr.*, **41**, 481 (1947).
(14) R. H. Mazur, B. W. Ellis, and P. S. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).

Ketenes. XII. Structure of Ketene–Enamine Cycloadducts[1]

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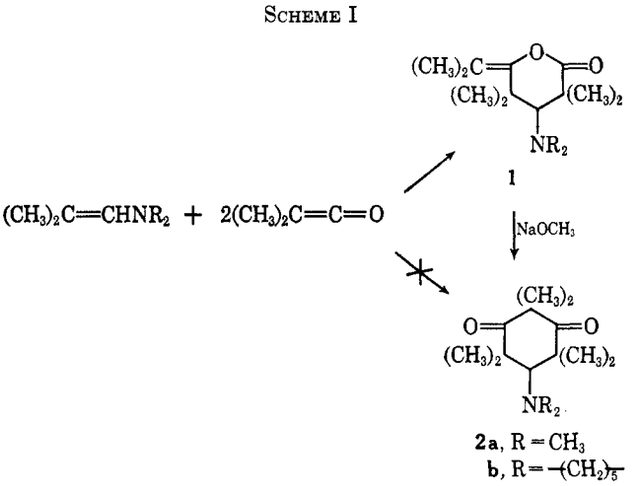
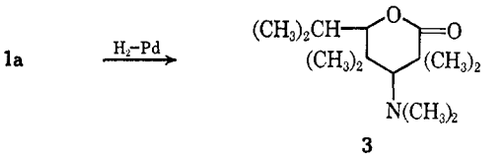
In an earlier publication we reported the synthesis of 2:1 cycloadducts from dimethylketene and isobutenylamines.[3] The aminocyclohexanedione structures (**2**) assigned to these products were in error; the compounds are actually enol lactones (**1**). (See Scheme I.)

SCHEME I



The correct structures were indicated by the chemical shifts for an isopropylidene group (singlets at 1.70 and 1.81 ppm which correspond to values noted in recent work on other cycloadducts of dimethylketene[4–7]). Further evidence for the presence of the isopropylidene group was obtained by catalytic hydrogenation to **1a** to the saturated δ-lactone (**3**).



When heated with sodium methoxide, **1a** and **1b** rearranged easily, in a manner analogous to the base-catalyzed rearrangement of compounds with similar

(1) Paper XI in this series: E. U. Elam, *J. Org. Chem.*, **32**, 215 (1967).
(2) To whom all correspondence should be addressed.
(3) R. H. Hasek and J. C. Martin, *J. Org. Chem.*, **28**, 1468 (1963).
(4) J. C. Martin, V. A. Hoyle, Jr., and K. C. Brannock, *Tetrahedron Letters*, 3589 (1965).
(5) J. C. Martin, K. C. Brannock, and R. H. Meen, *J. Org. Chem.*, **31**, 2966 (1965).
(6) H. U. Hostettler, *Tetrahedron Letters*, 1941 (1965).
(7) R. N. Pratt, S. A. Proctor, and G. A. Taylor, *Chem. Commun.*, 574 (1965).