Acid-Catalyzed Cyclization of Alkoxyacryloylureas to 2,4(1*H*,3*H*)pyrimidinediones

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1-Phenylthymine and the carbocyclic analog of thymidine were obtained in yields of 84-87% by cyclizing the appropriate 3-methoxy-2-methylacryloylureas in dilute sulfuric acid. High yields of 1-phenylthymine also resulted when the cyclization was carried out in trifluoroacetic acid, in acetic acid containing toluenesulfonic acid (TSA), or by fusion of the urea with a catalytic amount of TSA. In comparison, the typical aqueous-alkali catalyzed cyclizations gave lower yields of the two thymines, and cleavage of the acryloylureas was shown to occur. However, cyclization in concentrated aqueous ammonia produced high yields of both thymine derivatives.

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Cyclization of 3-(alkoxy)acrylamide and 3-(substituted-amino)acrylamide derivatives is the final step of a versatile synthesis (Chart 1), devised by Shaw and coworkers

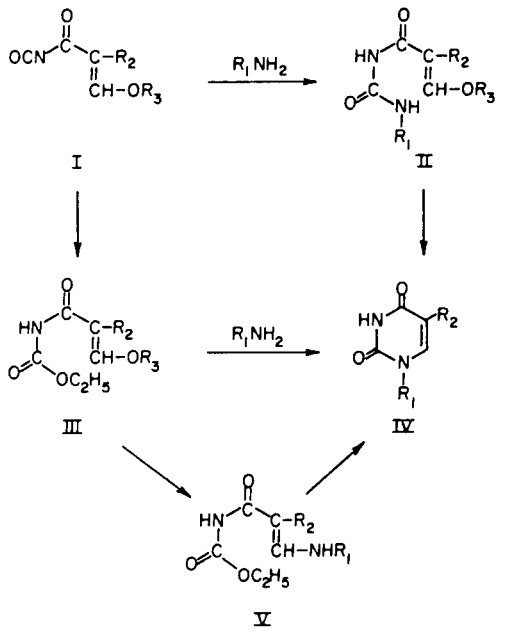


CHART I

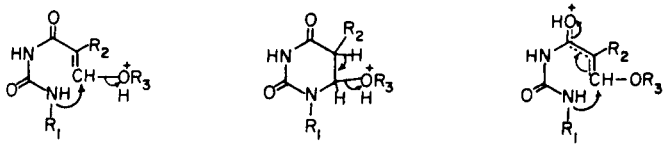
(1-17), of 1-substituted thymines, uracils, and other 2,4(1*H*,3*H*)pyrimidinediones (IV). One of the pathways comprising this method consists of the formation of an acryloyurea (II) from the reaction of an acryloyl isocyanate (I) with an appropriate amine (R_1NH_2) and subsequent cyclization of the acryloylurea (8, 11, 13). 2-Thioxo-4(1*H*)pyrimidinones (2-thiouracils and 2-thiothymines) have been prepared similarly *via* acryloylthioureas (7, 9, 10, 16). Cyclization of the ureas (II) and thioureas has been effected in basic media provided by aqueous alkali or ammonia (7, 8, 10, 11, 13, 14, 16), a tertiary amine in an organic solvent (9), or the amine (in excess, in water, or in an organic solvent) required to introduce the desired group (R_1) at position 1 of the pyrimidine ring (8, 10, 15).

An alternative - and more frequently used - pathway consists of the formation of an acryloylurethane (III) and treatment of the urethane with the appropriate amine in a basic environment such as aqueous alkali or carbonate (4-6, 12, 14,16), aqueous ammonia (1, 6, 12), aqueous amines (1, 2, 4, 6, 10, 12), an amine in an organic solvent (2, 4, 9, 16), or an alcohol solution of an alkoxide (16, 17). The intermediate between the urethane (III) and the pyrimidine (IV) appears, in general, to be a 3-(amino)acryloylurethane (V) (1, 2, 4, 6, 12, 16, 18, 22), which may be isolated and cyclized, although a urea (II) may also be an intermediate between III and IV (8).

When the R_2 group is electron-withdrawing (*e.g.*, CN, $-SO_2R$, $-COCH_3$, -COOR), 2,4(1*H*,3*H*)pyrimidinediones (IV) appear to be formed facilely (and sometimes in high yields (4, 6, 12, 18-20)) by base-catalyzed reaction of alkoxy-acryloylurethanes (III) with an amine or by thermal or base-catalyzed cyclization of 3-(amino)acryloylurethanes (V). When the R_2 group is a methyl group or hydrogen, conversion of alkoxyacryloylurethanes (III)

to thymines and unsubstituted uracils appears to be less facile (5, 21), although yields of 50-70% have been recorded (22-24). Similarly, although minor modifications of the base-catalyzed procedures have occasionally afforded good yields (25, 26) of thymines and unsubstituted uracils (R = CH_3 or H) from ureas (II), yields from typical procedures have frequently been modest or low (11, 13, 27-35). Probably for this reason, it has been suggested (16) that formation of 5-cyano- or 5-(ethoxycarbonyl)uracil derivatives, hydrolysis of these derivatives to uracil-5-carboxylic acids, and decarboxylation of the acids constitute a valuable route to uracil nucleosides (*cf.* 19). Prior formation of the anion of the urea by potassium *t*-butoxide in *t*-butanol and pyrolysis of the dry potassium salt have also been recommended (21) as improved modifications of the cyclization step.

Initial attempts to prepare the carbocyclic analog of thymidine by cyclization of the urea (VIb) in aqueous alkali gave low yields, and attempts to prepare a related thymine (1-[3-(hydroxymethyl)cyclopentyl]thymine) by the pyrolysis procedure (21) or by reaction of the amine with the required alkoxyacryloylurethane (III) were not encouraging. It seemed plausible that cyclization of alkoxy-acryloylureas (II) might be subject to acid catalysis for one, or more, of the following reasons: (*a*) protonation of the alkoxy group should facilitate expulsion of this group as the alcohol either in a direct displacement reaction (A, Chart 2) or in an elimination from a 5,6-dihydro-

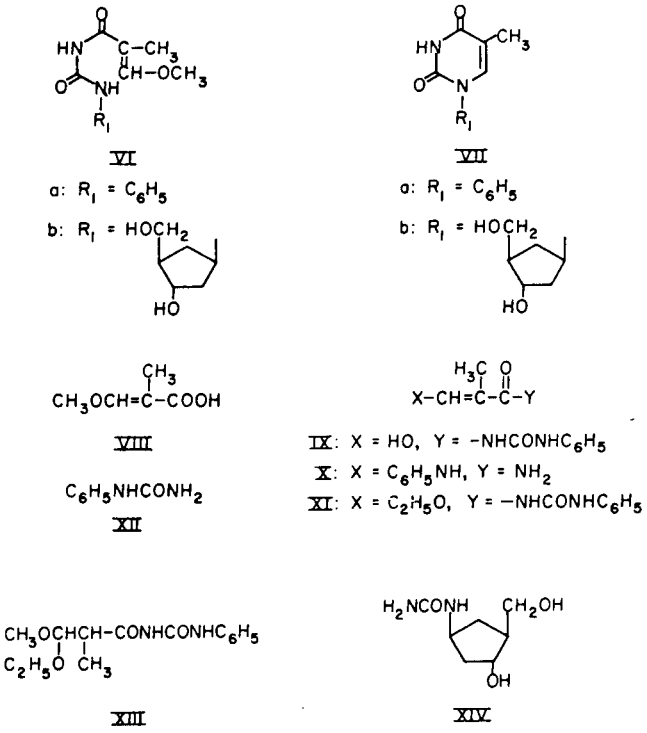


A B C

CHART 2

pyrimidine (B) formed by addition of the -NHR_1 group to the acryloyl moiety (14, 15); (*b*) protonation of the acryloyl carbonyl group, if this site is preferred, should also facilitate addition of the -NHR_1 group to the unsaturated carbonyl system (C); or (*c*) acidic hydrolysis of the enol ether might give an aldehyde (or hydroxymethylene) group, which could undergo condensation with the -NHR_1 group.

The formation in acidic media of 1-phenylthymine (VIIa), a model compound unencumbered by hydroxyl groups, was investigated, and acid catalysis was first attempted by fusing the phenylurea (VIa) with 0.15 equivalent of 4-toluenesulfonic acid (TSA). The fusion mixture (150-155°) solidified within thirty minutes; and, after a two-hour reaction period, 1-phenylthymine was isolated in 89% yield. In order to determine whether acidic conditions were essential for cyclization under the reaction conditions, the following experiments were performed. First, the urea was kept in the molten state at the same temperature (150-155°) and for the same period of time. Solidification did not occur, and the phenylurea (VIa) was recovered. Secondly, the urea was kept molten at 150-155° for three hours and TSA (0.15 equivalent) was then added. The mixture solidified within 15 minutes after the addition of TSA, and 1-phenylthymine was isolated. Despite the presence of unprotected hydroxyl groups, VIIb was also obtained, in modest yield, by fusing VIb with TSA.



VI: a: $R_1 = C_6H_5$; b: $R_1 = HOCH_2$

VII: a: $R_1 = C_6H_5$; b: $R_1 = HOCH_2$

VIII: $CH_3OCH{=}C(CH_3){-}COOH$

IX: X = HO, Y = $-NHCONHC_6H_5$

X: X = C_6H_5NH, Y = NH_2

XI: X = C_2H_5O, Y = $-NHCONHC_6H_5$

XII: $C_6H_5NHCONH_2$

XIII: $CH_3OCH(C_2H_5O)CH(CH_3)-CONHCONHC_6H_5$

XIV

CHART 3

Acid-catalyzed cyclization of VIa in several solvents was then investigated by examining aliquots of reaction mixtures by thin-layer chromatography (tlc). 1-Phenylthymine formed slowly in refluxing solutions of VIa and TSA (0.1-0.2 equivalent) in ethanol, dioxane, or tetrachloroethene. Some VIa remained and several other components were present, in addition to VIIa, after 26 and 48 hours, respectively, in refluxing ethanol or dioxane. In refluxing tetrachloroethene, some VIa also remained after 26 hours, but impure VIIa was isolated in 74% yield. The results were much improved when the cyclization of VIa was allowed to proceed in refluxing acetic acid containing 0.2 equivalent of TSA or in trifluoroacetic acid alone; pure 1-phenylthymine (VIIa) was isolated in

yields of 75% and 77% (36), respectively, Finally, cyclization of 3-(alkoxy)acryloylureas (II) in refluxing, dilute sulfuric acid proved to be an excellent procedure for preparing 1-substituted thymines. Both 1-phenylthymine (VIIa) and the carbocyclic analog (VIIb) of thymidine were obtained in yields of 84-87% by heating the ureas (VIa and VIb) in refluxing 2 *N* sulfuric acid for 3.5 hours (37). Further investigation showed that cyclization of VIb by the same procedure in 0.1 *N* sulfuric acid afforded a 79% yield of pure thymidine analog (VIIb). In contrast, cyclization of VIb to VIIb proceeded too slowly in refluxing distilled water (*p*H 5.6) to be of practical value; after 72 hours, the recovered urea (VIb) contained small amounts of VIIb and 3-methoxy-2-methyl-2-propenoic acid (VIII).

During the course of this investigation of acid catalysis, the ureas VIa and VIb were also cyclized in the usual basic media. Cyclization of VIa in an aqueous 2 *N* sodium hydroxide-ethanol (1:1) solution, the method reported (8) for 1-phenyluracil, gave 1-phenylthymine in an apparent yield of 61%, but the molar absorptivity of this material was greatly enhanced. By preparative tlc, impure VIIa was isolated in 50% yield, and a small amount of material with similar, but intense, uv absorption maxima was obtained from an impurity band. The mass spectrum of the latter material included peaks corresponding to the molecular ions of compounds IX, X, phenylurea (XII), and aniline (38). Cyclization of VIa in 2 *N* sodium hydroxide without added ethanol, the method reported (8) for this compound, afforded a 63% yield of pure VIIa. There was also mass spectral evidence for the presence of some unreacted VIa and for the formation of the propenoic acid (VIII), phenylurea (XII), and aniline during this cyclization.

As mentioned at the outset, this type of cyclization gave a low yield of VIIb. After urea VIb had been heated in refluxing aqueous sodium hydroxide (*N*) for one hour, applying the isolation procedure (continuous liquid extraction with ethyl acetate) that produced a high yield of VIIb after cyclization of VIb in sulfuric acid did not afford pure VIIb. A thymine (VIIb) fraction and 3-methoxy-2-methyl-2-propenoic acid (VIII) were separated by preparative thin-layer chromatography and identified by mass spectral and tlc analyses. The mass spectrum of the thymine (VIIb) fraction indicated that this fraction also contained urea XIV, and the yield of VIIb was estimated from uv data to be about 10%. Cyclization of VIb with 1 *N* sodium hydroxide was then repeated, and the isolation procedure was altered in order to liberate more VIII from its sodium salt and to achieve better separation of the three major products. From two uv-detectable bands on a preparative tlc plate, VIII and impure VIIb were isolated and identified, as before, by their mass spectra and by tlc alongside authentic specimens. A third fraction from a band at the origin, detectable by permanganate (on a similar small plate) but not by uv light, was shown by mass spectral analysis to be predominantly cyclopentylurea XIV. The yields of VIII and VIIb were 53% and 15%, respectively, and the yield of XIV, containing trace amounts of VIIb and VIII, was 69%. The formation of VIII during an attempt to prepare thymine from 3-methoxy-2-methylacryloylurea in 2 *N* sodium hydroxide has also been reported (8). These observations indicate that cleavage of alkoxyacryloylureas (II) in aqueous alkali may limit the yields of pyrimidinediones (IV) unless the cyclization is especially facile, as when R_2 of II is electron-withdrawing.

Alkoxyacryloylureas have been cyclized in dilute ammonia or in 15 *N* ammonia alone or diluted with ethanol. In general, yields were low, moderate, or unreported (8, 27, 28, 31, 33), but a high yield resulted (26) from a cyclization in ethanol-15 *N* aqueous ammonia (1:1). Very little cyclization occurred when VIa was treated with 15 *N* ammonia or with ethanol-15 *N* ammonia (1:1) by procedures similar to the sulfuric acid-catalyzed cyclization of VIa. The ratios of VIa to VIIa were estimated by NMR determinations to be about 12:1 and 6:1, respectively, after treatment of VIa with ammonia or ethanol-ammonia for 3.5 hours. Cyclization in ammonia alone may have been slowed by the sparing solubility of VIa, but the latter compound dissolved in the ethanol-ammonia solution. The mass spectrum of material isolated after the ethanol-ammonia treatment indicated the formation of small amounts of products (XIII, XI) resulting from the addition of ethanol to the acryloyl moiety of VIa and replacement of the methoxy group by an ethoxy group. However, treatment of VIa with 15 *N* ammonia in a bomb for a longer period gave a 92% yield of pure VIIa; there was also mass spectral and tlc evidence of slight cleavage of VIa to phenylurea (XII). Finally, treatment of urea VIb in refluxing 15 *N* ammonia also produced VIIb in high (87%) yield.

The experiments performed with VIa and VIb show that acid-catalyzed cyclization of alkoxy-acryloylureas is a convenient and high-yielding procedure for the preparation of 1-substituted-2,4(1*H*,3*H*)pyrimidinediones that are stable in dilute acid. They also indicate that this method is superior to the typical procedures for cyclization in aqueous alkali; yields from alkali-catalyzed cyclization may be limited by competing reactions, particularly cleavage of the ureas (II). However, cyclization in concentrated ammonia may, under the proper conditions, give excellent results. Cyclization of the appropriate ureas (II) in refluxing dilute sulfuric acid has been applied to the preparation of several other 1-substituted-2,4(1*H*,3*H*)-pyrimidinediones including the carbocyclic analogs of

uridine, 2'-deoxyuridine, and ribofuranosylthymine.

EXPERIMENTAL

General.

Unless otherwise stated, decomposition and melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus; those labelled "KH" were determined on a Kofler Heizbank apparatus (gradiently heated bar). Ultraviolet spectra (uv) were recorded with a Cary Model 17 or a Cary Model 14 spectrophotometer, and maxima are reported in nanometers. Solutions for ultraviolet determinations were prepared by diluting a 5-ml. aliquot of an ethanol solution to 50 ml. with 0.1 *N* hydrochloric acid, phosphate buffer (*p*H 7), or 0.1 *N* sodium hydroxide; absorption maxima are reported as being determined in 0.1 *N* hydrochloric acid, at *p*H 7, or in 0.1 *N* sodium hydroxide, respectively. Infrared spectra (ir) were recorded with Perkin-Elmer Model 521 or 621 spectrometers from samples in potassium bromide disks. Mass spectral data were taken from low resolution spectra determined at 70 eV with a Varian MAT Model 311A spectrometer equipped with a combination electron-impact, field-ionization, and field-desorption ion source. Unless otherwise indicated, the samples were introduced in a direct probe at 20°. Nmr spectra were determined with a Varian Model T-60A spectrometer for observing proton resonance at 60 MHz. Thin-layer chromatography (tlc) was performed on plates of silica gel, either Silica Gel H (39) or Silica Gel GF (40). Unless indicated otherwise in parentheses, tlc was performed as follows: (a) when Silica Gel H (SGH) was used, developed plates were examined with uv light (254 nm.) both before and after spraying with an optical whitening agent (Ultraphor WT, BASF Colors and Chemicals, Inc., Charlotte, N. C.); (b) when Silica Gel GF (SGF) was used, developed plates were examined by uv light (254 nm.) only; (c) tlc of all specimens of VIIb and of all other samples obtained during studies of the cyclization of VIb to VIIb was performed on plates of Silica Gel GF, and the developing solvent was 9:1 chloroform-methanol. Other pertinent information (amount applied, developing solvent, other methods of detection) is given parenthetically at the appropriate places in the experimental procedures. Preparative tlc was performed on Silica Gel 60 F-254 by E. Merck, precoated PLC plates, 2 mm. thickness, EM Laboratories, Elmsford, N. Y.

Cyclization of Phenylurea VIa to 1-Phenylthymine (VIIa).

A. Fusion with 4-Toluenesulfonic Acid.

(a) A mixture of 225 mg. (0.96 mmole) of 1-(3-methoxy-2-methylacryloyl)-3-phenylurea (VIa, m.p. 144°, reference 8) and 25 mg. (0.15 equivalent) of fused 4-toluenesulfonic acid was heated under a current of nitrogen at 150-155° for 2 hours. (The mixture melted initially, but solidified after 15-30 minutes of heating.) The solid was triturated with water, collected by filtration, washed with water, and dried *in vacuo* at 56°, yield, 172 mg. (88.7%), m.p. 199° (KH) [literature (8) m.p. 199°]; tlc, 1 spot (SGH, 40 mcg. 97:3 chloroform-methanol, detection by uv light, uv-Ultraphor WT, and basic permanganate spraying); ir and uv spectra identical with those of a recrystallized specimen; uv molar absorptivities (ϵ) 92-94% of those of the recrystallized specimen (below). Recrystallization of the beige solid from water afforded white needles, m.p. 199° (KH), 200-204° (capillary inserted at 120°, 3°/minute); uv max 273 (ϵ 11,700) in 0.1 *N* hydrochloric acid and at *p*H 7, 273 (ϵ 9,450) in 0.1 *N* sodium hydroxide; ir: (1800-1500 cm^{-1} region only) 1690, 1680, 1655, 1645, 1590, 1500; mass spectrum: (direct-inlet temperature, 20°) m/e 202 (M^+), 159 (M - NHCO), 130, 104, 77 (phenyl).

Anal. Calcd. for $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.99; N, 13.86. Found: C, 65.16; H, 4.90; N, 13.79.

(b) The phenylurea (25 mg.) was heated alone for 2 hours as described above. The molten material did not solidify until it was cooled, and tlc (SGH, 40 mcg., chloroform) showed only VIa. Recrystallization of the resolidified material from ethanol afforded 20 mg. (80%) of white crystals identified by ir and m.p. (143°, KH) as VIa.

(c) The phenylurea (225 mg.) was heated as described above for 3 hours, 25 mg. of TSA was added, and the mixture was kept at 150-155° for an additional hour. Tlc of a specimen of the molten material removed just before the addition of TSA revealed only VIa. The mixture solidified within 15 minutes after the addition of TSA, and tlc (SGH, 99:1 chloroform-methanol) of a specimen removed 1 hour after the addition of TSA showed only VIIa, TSA, and a trace impurity. Trituration of the yellow solid with ethanol (5 ml.) yielded 57 mg. of white crystalline solid, identified as VIIa by m.p. (199°, KH) and by its ir spectrum; recrystallization of the filtrate residue from water afforded 56 mg. of VIIa as white crystals (m.p. 199°, KH). The yield (58.5%) was reduced by sublimation of some of VIa during the 3-hour period of heating prior to the addition of TSA.

B. In Organic Solvents.

The course of the acid-catalyzed cyclization of VIa to VIIa in several solvent media was monitored by tlc.

(a) A solution of VIa and 0.11 equivalent of TSA was heated in refluxing ethanol. Tlc (SGH, 99:1 chloroform-methanol) of aliquots showed that a small amount of VIIa was present after 2 hours and after 6 hours, but most of VIa remained. After 26 hours, VIIa appeared to be the principal component, but several other compounds, including VIa, were also present.

(b) In a refluxing dioxane solution of VIa and 0.16 equivalent of TSA, a trace amount of 1-phenylthymine was detected by tlc (SGH, 95:5 chloroform-methanol) after 1.5 hours, but VIa remained the principal component after 48 hours and small amounts of several other components were also detected.

(c) A solution of VIa, 0.23 equivalent of TSA, and tetrachloroethene was heated under reflux for 26 hours at which time tlc (SGH, 99:1 chloroform-methanol) showed that some unchanged VIa, as well as VIIa, was present. The yellow solution, decanted from a black tar, deposited crystalline VIIa during cooling, yield, 74%, m.p. 195-196° (KH); identity established by ir spectrum; uv molar absorptivities (ϵ) were 86-88% of those of pure VIIa.

(d) A solution of 150 mg. (0.64 mmole) of VIa, 25 mg. (0.23 equivalent) of TSA, and 10 ml. of acetic acid was heated under reflux for 2 hours and concentrated *in vacuo* to a syrup. Evaporation of several portions of ethanol from the syrup followed by trituration with water produced a solid that was collected by filtration, washed with water, dried *in vacuo* at 56°, and identified as VIIa by tlc (SGH, 98:2 chloroform-methanol) and m.p. (199°, KH): yield, 98 mg. (75%). Additional VIIa, as well as impurities, was detected in the filtrate by tlc.

(e) Pure VIIa (m.p. 199°, KH; tlc, 1 spot) was obtained in the same way from an identical solution of VIa in trifluoroacetic acid without TSA: yield, 100 mg. (77%). In addition, only VIIa was detected by tlc (SGH, 98:2 chloroform-methanol) in the filtrate (36).

C. In 2 *N* Sulfuric Acid.

(a) A mixture of 1.844 g. of VIa in 150 ml. of 2 *N* sulfuric acid was heated under reflux for 3.5 hours and allowed to stand at room temperature overnight. The mixture became homogeneous

within 30 minutes. The cooled solution deposited white needles that were collected by filtration, washed thoroughly with water, and dried *in vacuo* at 56°, yield, 1.34 g. (84%) (37), m.p. 199° (KH), 200-203° (capillary, inserted at 120°, 3°/minutes); tlc, 1 spot (SGH, 80 mcg., 98:2 chloroform-methanol); uv max 273 (ϵ 11,700) in 0.1 *N* hydrochloric acid, 273 (ϵ 11,950) at *p*H 7, 274 (ϵ 9,600) in 0.1 *N* sodium hydroxide. The ir and uv spectra and the thin-layer chromatogram showed that this material was comparable to the recrystallized, analytically pure specimen described above (TSA fusion method).

(b) A similar experiment, on a smaller scale, allowed to proceed for 2 hours at reflux afforded a yield of 86% of VIIa (m.p. 199°, KH); tlc detected a trace of unchanged VIa. Only VIIa was detected in the filtrate.

D. In Aqueous Base (41).

(a) A solution of 104 mg. of VIa, 2 ml. of 2 *N* sodium hydroxide, and 2 ml. of ethanol was heated under reflux for 1 hour, cooled to room temperature, and acidified to *p*H 2.5 with 2 *N* hydrochloric acid (42). The homogeneous solution was concentrated *in vacuo* to about half of the original volume; a white precipitate was then filtered from the chilled mixture, washed with water, and dried *in vacuo* at 56°: weight, 55 mg. (61% calculated as pure VIIa); m.p. 183-187°; uv max 275 in 0.1 *N* hydrochloric acid and 273 in phosphate buffer and in 0.1 *N* sodium hydroxide. The crude product contained impurities that remained at the origin on a tlc plate (SGH, 80 mcg., 98:2 chloroform-methanol) and greatly enhanced the molar absorptivity of VIIa. Mass spectral peaks at m/e 220, 176, 136, and 93 were prominent in addition to the stronger peaks arising from VIIa (*e.g.*, m/e 202, 159) (38). Part of the crude product (44 mg.) was chromatographed in chloroform-methanol (98:2) on a preparative tlc plate. Extraction of the faster-moving band with chloroform-methanol (1:1, 2 x 25 ml.) and evaporation of the solvent left 36 mg. of impure VIIa (m.p. 193-195°, adjusted yield = 50%), identified by tlc (SGF, 98:2 chloroform-methanol) and by its mass spectrum (which still included weak impurity peaks).

Extraction of the band at the origin with ethyl acetate and evaporation of the solvent left 7 mg. of a white solid: mass spectrum (direct-inlet temperature 20°) m/e 220 (presumably M^+ of IX), 202 (weak, M^+ of VIIa), 176 (presumably M^+ of X), 149, 136 (presumably M^+ of XII), 119, 93 (presumably M^+ of aniline), 77 (phenyl); uv max (ϵ calculated for molecular weight = 220) 278 (ϵ 23,900) in 0.1 *N* hydrochloric acid, 271 (ϵ 22,600) in phosphate buffer (*p*H 7), 271 (ϵ 22,200) in 0.1 *N* sodium hydroxide.

(b) A mixture of 410 mg. of VIa and 5 ml. of 2 *N* sodium hydroxide was stirred at 80° until VIa had dissolved (55 minutes), cooled to room temperature, and acidified to *p*H 3.5 (43). A white solid, which precipitated during the acidification, was filtered from the chilled mixture, washed with water, and dried *in vacuo* at 56°: weight, 238 mg. (67% yield calculated as VIIa); m.p. 197-203°. The mass spectrum of gummy material extracted with ethyl acetate from the filtrate residue included strong peaks corresponding to phenylurea (XII) and aniline (m/e 136 and 93, respectively) in addition to peaks due to VIIa and weaker peaks corresponding to VIa (m/e 234) and VIII (m/e 116). Since tlc (SGF, 40 mcg., 99:1 chloroform-methanol) of the white precipitate revealed an impurity at the origin and the mass spectrum included weak peaks corresponding to XII and aniline, it was recrystallized from water, yield, 63%, m.p. 199-203°; mass spectrum and tlc identical with those of the analytical sample.

(c) A suspension of 234 mg. (1 mmole) of VIa in 10 ml. of 15 *N* aqueous ammonia was heated under reflux for 3.5 hours, allowed to stand at room temperature overnight, and evaporated to dryness *in vacuo*, weight, 233 mg., m.p. 142-144° with premature softening at 130-135°. The pmr spectrum indicated that the ratio of VIa to VIIa was about 12:1, and tlc (SGF, 80 mcg., 99:1 chloroform-methanol) confirmed that this material was VIa containing a small amount of VIIa and two slower-moving trace components.

(d) Experiment *c* was repeated except for the facts that 10 ml. of ethanol-15 *N* ammonia (1:1) was used and the reaction mixture was homogeneous. The pmr spectrum of the reaction-mixture residue indicated that the ratio of VIa to VIIa was about 6:1 and that small amounts of other components were present, and tlc (SGF, 80 mcg., 99:1 chloroform-methanol) confirmed that the residue consisted of VIa containing some VIIa and four, or more, impurities. The mass spectrum included (in addition to peaks due to VIa, VIIa, and their fragments) peaks of m/e 280 and 248, which correspond, respectively, to the molecular ions of the product (XIII) of addition of ethanol to VIa and the product (XI) of replacement of the methoxy group by an ethoxy group.

(e) A mixture of 234 mg. of VIa and 10 ml. of 15 *N* aqueous ammonia was heated in a stainless steel bomb at 100° for 20 hours. Concentration of the solution to dryness *in vacuo* left a white solid, weight, 201 mg., m.p. 195-198° with premature softening at 192-195°. The mass spectrum of this material was very similar to that of pure VIIa, but a weak peak of m/e 136 (XII) indicated that slight cleavage of VIa had occurred, and a weak spot at the origin of a tlc plate (SGF, 40 mcg., 99:1 chloroform-methanol) supported this conclusion. Recrystallization of this material from water gave white needles (VIIa), yield, 92%, m.p. 200-204°; tlc, 1 spot (SGF, 80 mcg., 99:1 chloroform-methanol).

Cyclization of VIb to (1α,3β,4α)-(±)-[3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4(1*H*,3*H*)pyrimidinedione (VIIb).

A. In Sulfuric Acid.

(a) A solution of 1.605 g. of VIb (44) in 60 ml. of 2 *N* sulfuric acid was heated under reflux for 3.5 hours, cooled, and neutralized with aqueous sodium hydroxide to *p*H 5.6. The aqueous solution was saturated with sodium chloride and subjected to continuous liquid-liquid extraction with ethyl acetate. White crystals were filtered from the cooled ethyl acetate extract, washed with ethyl acetate, and dried *in vacuo* at 78° for 2 hours, weight, 950 mg., m.p. 219-221.5° dec. (inserted at 180°, 2-3°/minute); tlc, 1 spot (SGH, 80 mcg., 9:1 chloroform-methanol, detection by uv light, uv-Ultraphor WT, and basic potassium permanganate spray); uv max 273 (ϵ 10,400) in 0.1 *N* hydrochloric acid and at *p*H 7, 272 (ϵ 8,100) in 0.1 *N* sodium hydroxide; ir: (1800-1450 cm^{-1} only) 1675, 1630, 1510 weak, 1472; mass spectrum: (45) (direct-inlet temperature, 20°) m/e 240 (M^+), 223 (M - OH), 222 (M - H_2O), 210, 203, 191, 183, 181, 153 (Th + C_2H_4), 127 (Th + 2H), 126 (Th + H), 110, 96.

Anal. Calcd. for $C_{11}H_{16}N_2O_4$: C, 54.99; H, 6.71; N, 11.66. Found: C, 54.82; H, 6.82; N, 11.32.

Continuous liquid-liquid extraction of the aqueous solution with a second portion of ethyl acetate afforded 290 mg. (total yield, 87.6%) identical by m.p. (219-222° dec.) and by ir and uv spectra with the first portion.

(b) A solution of 500 mg. of VIb in 18.5 ml. of 0.1 *N* sulfuric acid was heated under reflux for 3.5 hours. (Tlc of an aliquot removed after 2 hours indicated that some VIb remained.) The cooled solution was neutralized with 2 *N* sodium hydroxide to

*p*H 5.6 and, then, subjected to continuous liquid-liquid extraction with ethyl acetate for 24 hours. The concentrated extract deposited white crystals that were washed with ethyl acetate and dried *in vacuo* at 56°, yield, 348 mg. (79%), m.p. 216-219° dec. (inserted at 180°, 3°/minute); uv and mass spectra and tlc were identical with those outlined above.

B. In Water.

A solution of 100 mg. of VIb in 5 ml. of water was heated under reflux for 72 hours. Tlc of aliquots removed during this period showed that small amounts of VIIb and 3-methoxy-2-methyl-2-propenoic acid (VIII) were being formed. The residue obtained by evaporating the solution to dryness was shown by tlc and by the mass spectrum (46) to be VIb (m/e 272) containing small amounts of VIIb and VIII (m/e 116, 101); pure specimens of VIb, VIIb, and VIII were spotted on the tlc plate alongside the residue for positive identification.

C. By Fusion with TSA.

A mixture of 272 mg. (1 mmole) of VIb (m.p. 139-140°) and 19 mg. (0.11 mmole) of fused 4-toluenesulfonic acid was heated at 138-140° for 40 minutes under a nitrogen atmosphere. A solution of the dark mixture in methanol was filtered to remove a small amount of black solid and concentrated to dryness. A solution of the residual brown gum in water (8 ml.) was neutralized to *p*H 6.6 with 0.1 *N* sodium hydroxide, saturated with sodium hydroxide, and extracted continuously with ethyl acetate for 18 hours. The residue obtained by evaporating the ethyl acetate was chromatographed in chloroform-methanol (9:1) on a preparative tlc plate. The band containing VIIb was leached with warm methanol (2 x), the methanol was evaporated, and the residue was leached with ethanol. Evaporation of the ethanol left 110 mg. (46% yield) of VIIb identified by m.p. (218-220° dec.), tlc (1 spot when 80 or 120 mcg. applied), and uv spectra.

D. In Aqueous Base.

(a) A solution of 495 mg. of VIb in 16 ml. of 1 *N* sodium hydroxide was heated under reflux for 1 hour. The reaction solution was cooled to room temperature, neutralized to *p*H 5.6 with concentrated sulfuric acid, saturated with sodium chloride, and extracted continuously with ethyl acetate (50 ml.) for 18 hours. No VIIb precipitated from the ethyl acetate extract, in contrast to the precipitation of VIIb at this stage after cyclization of VIb in sulfuric acid. Tlc (47) showed that VIIb and 3-methoxy-2-methyl-2-propenoic acid (VIII) were present in both the ethyl acetate extract and in the aqueous layer. Accordingly, organic components were isolated from the aqueous layer as follows: the aqueous layer was concentrated to dryness *in vacuo* after the *p*H had been adjusted to 7, the residue was leached with ethanol (3 x 20 ml.), the ethanol extract was concentrated to dryness, the residue was redissolved in ethanol (10 ml.), the mixture was filtered, and the filtrate was concentrated to dryness. The residue, combined with the residue from the ethyl acetate extract, was chromatographed in chloroform-methanol (9:1) on a preparative tlc plate. The faster-moving band was leached from the silica gel with warm ethanol. Evaporation of the ethanol left 76 mg. of VIII, identified by tlc alongside authentic VIII (7) and by the mass spectrum: m/e 116 (M^+), 101 ($M - CH_3$), 99 (M - OH), 71 (M-COOH). A gummy white solid (108 mg.) was obtained in the same way from the slower-moving band or bands. The mass spectrum and tlc (47) showed that this material contained VIIb, and comparison of its mass spectrum (48) with mass spectra of pure VIIb and VIb revealed significant peaks indicative of the presence of urea XIV: m/e 174 (M^+) 157 ($M - OH$ or NH_3), 156 ($M - H_2O$), 143 ($M - CH_2OH$), 130 ($m - H_2NCO$), 125 ($M - CH_2OH-H_2O$), 115 ($M - H_2NCONH$). Uv data indicated that this material was about 40% VIIb and, therefore, indicated that the yield of VIIb was about 10%.

(b) Experiment *a* above was repeated, but the isolation procedure was altered to liberate more of VIII from its salt and to separate all three of the major products. A solution of 125 mg. of VIb in 4 ml. of 1 *N* sodium hydroxide was heated under reflux for 1 hour, cooled to room temperature, acidified to *p*H 4 with 2 *N* sulfuric acid, and concentrated *in vacuo* to a gummy solid. Mass spectra (direct-inlet temperatures, 20° and 80°) of this material included molecular-ion and fragment peaks arising from VIIb, 3-methoxy-2-methyl-2-propenoic acid (VIII) (46), and cyclopentylurea XIV (48); tlc (40 or 80 mcg., detection by uv light only) confirmed the presence of VIIb and VIII. The gummy solid was slurried with ethanol (2 x 10 ml.), and the ethanol was evaporated. Tlc (40 or 80 mcg., detection by uv light and by basic permanganate spray) of the gummy residue (131 mg.) again revealed VIIb and VIII plus a third spot at the origin detectable by basic permanganate, but not by uv. An ethanol solution of the gummy residue was applied to a preparative tlc plate, and the plate was developed with chloroform-methanol (9:1). The two uv-detectable bands and the origin band were scraped from the plate and extracted separately with ethanol (3 x 25 ml.). Evaporation of ethanol from the extract of the band (R_f 0.6) farthest from the origin left 28 mg. (53% yield) of white solid, identified as VIII by tlc (40 or 80 mcg., detection by uv light and by basic permanganate spray) alongside an authentic specimen (7) and by its mass spectrum: m/e 116 (M^+), 101 ($M - CH_3$), 99 (M-OH), 71 (M-COOH). The ethanol extract of the second band (R_f 0.16) yielded 20 mg. (18%) of white, glassy solid identified by tlc and by its mass spectrum as impure VIIb. The uv molar absorptivities of this material were about 81% of those of pure VIIb and indicated, therefore, that the yield of VIIb was about 14-15%. Concentration of the extract from the origin band (R_f 0) left 55 mg. (69% yield) of colorless syrup. Tlc (40 or 80 mcg., detection by uv and by basic permanganate spray) in chloroform-methanol (3:1 and 5:1) revealed a major permanganate-detectable spot that moved differently from VIIb, VIII, and the cyclopentylamine used to prepare VIb. (Weak spots corresponding to trace amounts of VIIb and VIII and, possibly, the cyclopentylamine were also detected.) The mass spectrum of this fraction was in agreement with the structure of cyclopentylurea XIV: m/e 174 (M^+), 157 ($M - OH$ or NH_3), 156 ($M - H_2O$), 143 ($M - CH_2OH$), 130 ($M - H_2NCO$), 125 ($M - CH_2OH-H_2O$), 115 ($M - H_2NCONH$).

(c) A solution of 272 mg. of VIb in 15 ml. of 15 *N* aqueous ammonia was heated under reflux for 3.5 hours, allowed to stand at room temperature overnight, and concentrated to dryness *in vauco*. After several portions of ethanol had been evaporated *in vacuo* from the white residue, it was triturated with ethyl acetate (8 ml.), collected by filtration, washed with ethyl acetate, and dried *in vacuo* at 56°, yield, 223 mg. (93%), m.p. 218-221° dec., tlc, 1 spot (detection by uv light only). Molar absorptivities of uv spectra at *p*H 1, 7, and 13 indicated that this material was about 94% VIIb and that the yield, therefore, was about 87%.

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REFERENCES AND NOTES

(1) G. Shaw, *J. Chem. Soc.*, 1834 (1955).

(2) R. K. Ralph and G. Shaw, *ibid.*, 1877 (1956).

(3) M. R. Atkinson, G. Shaw, K. Schaffner, and R. N. Warrener, *ibid.*, 3847 (1956).

(4) M. R. Atkinson, G. Shaw, and R. N. Warrener, *ibid.*, 4118 (1956).

(5) M. R. Atkinson, M. H. Maguire, R. K. Ralph, G. Shaw, and R. N. Warrener, *ibid.*, 2363 (1957).

(6) M. R. Atkinson, G. Shaw, and G. Sugowdz, *ibid.*, 3207 (1957).

(7) G. Shaw and R. N. Warrener, *ibid.*, 153 (1958).

(8) G. Shaw and R. N. Warrener, *ibid.*, 157 (1958).

(9) G. Shaw, R. N. Warrener, M. H. Maguire, and R. K. Ralph, *ibid.*, 2294 (1958).

(10) G. Shaw and R. N. Warrener, *ibid.*, 50 (1959).

(11) R. K. Ralph, G. Shaw, and R. N. Naylor, *ibid.*, 1169 (1959).

(12) J. H. Dewar and G. Shaw, *ibid.*, 3254 (1961).

(13) J. H. Dewar and G. Shaw, *ibid.*, 583 (1962).

(14) J. H. Dewar and G. Shaw, *ibid.*, 1642 (1965).

(15) P. Lees and G. Shaw, *J. Chem. Soc., (C)*, 1519 (1968).

(16) N. J. Cusack, B. J. Hildick, D. H. Robinson, P. W. Rugg, and G. Shaw, *J. Chem. Soc., Perkin Trans. I*, 1720 (1973).

(17) N. J. Cusack, D. H. Robinson, P. W. Rugg, G. Shaw, and R. Lofthouse, *ibid.*, 73 (1974).

(18) S. Senda, K. Hirota, and J. Notani, *Chem. Pharm. Bull.*, **20**, 1380 (1972).

(19) J. T. Kusmierek and D. Shugar, *Acta Biochim. Pol.*, **17**, 259 (1970).

(20) J. Goerdeler and J. Gnad, *Chem. Ber.*, **98**, 1531 (1965).

(21) K. C. Murdock and R. B. Angier, *J. Org. Chem.*, **27**, 3317 (1962).

(22) J. Defaye, M. Naumberg, and T. Reyners, *J. Heterocyclic Chem.*, **6**, 229 (1969).

(23) H. De Koning and U. K. Pandit, *Rec. Trav. Chim.*, **90**, 874 (1971).

(24) F. M. Kaspersen and U. K. Pandit, *J. Chem Soc., Perkin Trans. I*, 1798 (1975).

(25) A. P. Martinez, W. W. Lee, and L. Goodman, *J. Med. Chem.*, **8**, 187 (1965).

(26) M. W. Logue and N. J. Leonard, *J. Am. Chem. Soc.*, **94**, 2842 (1972).

(27) T. Ukita, A. Hamada, and M. Yoshida, *Chem. Pharm. Bull.*, **12**, 454 (1964).

(28) K. Tokuyama and M. Katsuhara, *Bull. Chem. Soc. Japan*, **39**, 2728 (1966).

(29) J. Šmejkal, J. Farkaš, and F. Šorm. *Collect. Czech. Chem. Commun.*, **31**, 291 (1966).

(30) A. Kjaer, A. Knudsen, and P. O. Larsen, *Acta Chem. Scand.*, **15**, 1193 (1961).

(31) T. Naito, M. Hirata, T. Kawakami, and M. Sano, *Chem. Pharm. Bull.*, **9**, 703 (1961).

(32) T. Naito and M. Sano, *ibid.*, **9**, 709 (1961).

(33) T. Naito and T. Kawakami, *ibid.*, **10**, 627 (1962).

(34) M. Sano, *ibid.*, **10**, 308 (1962).

(35) H. Kaye, *Macromolecules*, **4**, 147 (1971).

(36) The yield of VIIa from the cyclization of VIa in trifluoroacetic acid was probably quantitative since only VIIa was detected in the filtrate by tlc.

(37) A trace of VIa was detected by tlc in VIIa isolated (in 86% yield) after a 2-hour cyclization in 2 *N* sulfuric acid; only VIIa was detected in the filtrate. After a 3.5-hour cyclization in 2 *N* sulfuric acid, the isolated VIIa (84% yield) was chromatographically homogeneous. Although the second filtrate was not examined, the results of these two experiments, considered together, suggest (a) that the longer time was required to complete the reaction and (b) that the yield of VIIa in the second experiment was probably quantitative.

(38) The filtrate from the original precipitate was not examined for these by-products or for VIII.

(39) Silica Gel H plates were made from Silica Gel H for tlc according to Stahl, without calcium sulfate binder, 10-40 microns, EM Reagents (E. Merck, Darmstadt, Germany), distributed by Brinkmann Instruments, Inc., Westbury, N.J.

(40) Silica Gel GF precoated thin-layer chromatography plates (fluorescent), 250 microns in thickness, were purchased from Analtech Inc., Blue Hen Industrial Park, Newark, Delaware, 19711.

(41) Most of the mass spectral peaks used to identify by-products of the base-catalyzed cyclization of VIa were insignificant in the spectra of VIa and VIIa. When peaks used to identify by-products in mixtures were also present in the spectra of VIa or VIIa, the identifying peaks were compared with molecular-ion and strong-fragment peaks of VIa and VIIa in the spectra of the mixtures. In this way, it could be observed unequivocally that the identifying peaks did not arise from the starting material or the product.

(42) This procedure was patterned after the published procedure (8) for the preparation of 1-phenyluracil.

(43) This procedure was patterned, insofar as possible, after the published procedure (8) for the preparation of 1-phenylthymine.

(44) Y. F. Shealy and C. A. O'Dell, unpublished.

(45) Th = the thyminyl moiety ($C_5H_5N_2O_2$).

(46) Since the peak of m/e 101 is strong in spectra of VIII, but is insignificant in spectra of VIb and VIIb, it was important in identifying VIII in mixtures. The peak of m/e 116 is insignificant in spectra of VIIb; it is present, but weaker than m/e 115, in spectra of VIb.

(47) During this experiment, thin-layer chromatograms were examined with uv light only; and, therefore, the cyclopentylurea (XIV) was not detected.

(48) The peaks of m/e 174, 157, 156, and 125 are insignificant in mass spectra of pure VIIb and VIb and, therefore, were diagnostic in identifying XIV in mixtures.