Steroids. XXI[1]*. The Oxidation and Hypobromous Acid Addition of Steroids by Means of Isocyanur Bromide*

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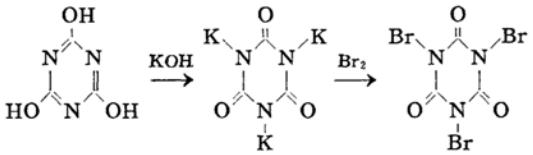
Recently it was shown that isocyanur chloride[2-5] is an effective reagent for oxidation[6] and hypochlorous acid addition[7,8] of steroids. The present investigation aims at elucidating the characteristics of isocyanur bromide, which has not yet been obtained in a pure state, towards oxidation, hypobromous acid addition and allylic bromination of steroids.

The synthesis of isocyanur bromide was attempted by Chattaway and Wadmore[2] about half a century ago. Adding bromine to a solution of cyanuric acid in a cold 5% solution of caustic potash, they obtained a pale yellow substance which rapidly decomposed on exposure to air, evolving bromine. They posturated that the substance was a bromine addition product of a bromoimino derivative of cyanuric acid, in which all the iminohydrogen atoms of the cyanuric acid were not substituted. The present author added bromine to an alkaline solution of cyanuric acid in the cold with swirling with hand, and obtained a similar substance, while, when bromine was carefully added with vigorous stirring at 0~3°C, a somewhat stable substance was obtained. A better result was obtained when the order of addition was reversed, namely, when the alkaline cyanuric acid solution was added to bromine[9]. It was necessary to remove

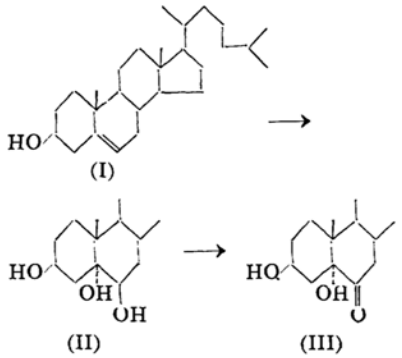
1) Part XX: K. Morita, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **78**, 1705 (1957).
2) F. D. Chattaway and J. M. Wadmore, *J. Chem. Soc.*, **1902**, 191.
3) C. H. G. Hands, F. R. White and J. W. C. Phillips, *J. Soc. Chem. Ind.*, **61**, 66 (1948); Brit. Pat., 634, 801; *Chem. Abst.*, **44**, 7356e (1950).
4) T. Ishii, S. Kanai and T. Ueda, *J. Soc. Org. Synth. Chem. Japan* (*Yuki Gosei Kagaku Kyokaishi*), **15**, 241 (1957).
5) Isocyanur chloride is also a bleaching and sterilizing agent. Cf. *Chem. Trade J.*, **140**, 884 (1957).
6) F. Mukawa, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **78**, 450 (1957).
7) Idem., ibid., **78**, 452 (1957).
8) S. Mori, K. Morita and F. Mukawa, *Proc. Japan Acad.*, **23**, 535 (1956).
9) Y. Yukawa and U. Suzuki (*Mem. Inst. Sci. Ind. Des. Osaka Univ.*, **10**, 190, (1952) stated that the addition of alkaline succinimide to a mixture of bromine and water was preferable for preparation of *N*-bromosuccinimide.

the moisture in the cake after filtration as fast as possible. The moisture lingering in the product markedly decreased the purity. The percentage of bromine in the product was determined by micro Carius method and by iodometry[4] with well coincident results. The purity of the product was 80~92%. In some cases the isocyanur bromide contained bromine in a percentage as low as 50% (cf. experimental part), but it should be noted that for oxidation and hypobromous acid addition, 50~70% isocyanur bromide was usable as well.



Isocyanur bromide is a white or slightly yellow crystalline powder. It has an odour resembling that of hypobromous acid. It does not melt below 300°C, but decomposes when heated by direct flame. It dissolves to some extent in water and in glacial acetic acid with hydrolysis. When it is added to hydrobromic acid, bromine is liberated. It liberates iodine from hydroiodic acid and from an aqueous solution of potassium iodide. When added to aqueous ammonia, a violent decomposition takes place.

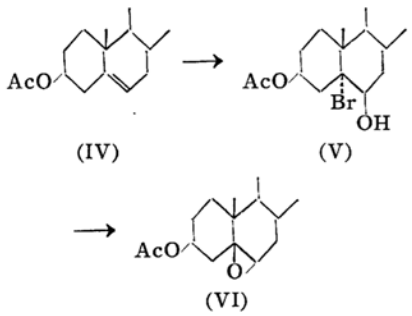
Mukawa[6] reported that the reaction of cholestane-3 β,5 α,6 β-triol (II) with isocyanur chlorid and pyridine in warm benzene furnished cholestane-3 β,5 α-diol-6-one (III) in 80% yield. Under similar conditions the starting material was recovered when isocyanur bromide was used instead of isocyanur chloride, while the oxidation took place smoothly to produce cholestane-3 β,5 α-diol-6-one (III) when *tert*-butanol was used as the solvent. Fieser et al.[10] reported that the oxidation of cholesterol (I) with *N*-bromosuccinimide and acetic acid in aqueous acetone produced cholestane-3 β,5 α-diol-6-one (III) and choleterol dibromide. Under nearly the same conditions except that isocyanur bromide was used in place of *N*-bromosuccinimide cholestane-3 β,5 α,6 β-triol (II) and cholesterol dibromide were obtained but cholestane-3 β,5 α-diol-6-one (III) was not detected. A similar result was reported by Ueno[11], who found that oxidation of cholesterol with *N*,*N*-dibromobenzenesulfonamide gave cholestane-3 β,5 α,6 β-triol (II). It is concluded that isocyanur bromide is an oxidizing agent milder than *N*-bromosuccinimide or isocyanur chloride.



Hypochlorous acid addition of 5-ene-steroids by means of isocyanur chloride and acetic acid in aqueous acetone is known to produce corresponding chlorohydrins in good yields[7,8]. Hypobromous acid addition of cholesteryl acetate (IV) by means of isocyanur bromide and acetic acide in the same solvent furnished 5 α-bromocholestane-3 β,6 β-diol 3-monoacetate (V) and cholesteryl acetate dibromide (VII) only in low yields. Fried and Sado[13] demonstrated that hypobromous acid addition of 4,9(11)-pregnadiene-17 α,21-diol-3,20-dione 21-acetate with *N*-bromoacetamide in dioxane containing dilute sulfuric acid gave 9 α-bromohydrocortisone acetate in only 48% yield, while in dioxane containing dilute perchloric acid, 9 α-bromohydrocortisone acetate was obtained in 80~90% yield[14]. Mori[15] reported that the reaction of cholesteryl acetate (IV) with *N*-bromoacetamide in ether containing dilute sulfuric acid gave no hypobromous acid addition product V, but gave cholesteryl acetate dibromide (VII). The present author obtained the bromohydrin (V) from cholesteryl acetate (IV) in 61% yield with *N*-bromoacetamide and dilute perchloric acid in dioxane and in 49% yield with isocyanur bromide and dilute perchloric acid in the same solvent. Ueno[12] reported

10) L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3938 (1949).
11) Y. Ueno, *J. Pham. Soc. Japan* (*Yakugaku Zasshi*), **72**, 1626 (1952).
12) Idem., ibid., **72**, 1622 (1952).
13) J. Fried and E. F. Sabo, *J. Am. Chem. Soc.*, **79**, 1136 (1957).
14) Perchloric acid has been employed on hydroxybromination of 9(11)-ene-steroids (H. B. Henbest et al., *J. Chem. Soc.*, **1955**, 2477; R. H. Lenhars and S. Bernstein, *J. Am. Chem. Soc.*, **77**, 6665 (1955)), of 11-ene-steroids (J. E. Herzig et al., ibid., **78**, 2017 (1956)), and of 5-ene-steroids (B. Ellis and V. Petrow, *J. Chem. Soc.*, **1956**, 4417). Cf. B. Loenken et al., *J. Am. Chem. Soc.*, **78**, 1738 (1956).
15) S. Mori, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **73**, 505 (1952).

the same hydroxy-bromination reaction with *N*-bromosuccinimide and with *N,N*-dibromobenzenesulfonamide and acetic acid in aqueous acetone, obtaining compound V in 15 and 37% yields, respectively[16]. Proof for the bromohydrin structure was obtained from its coversion into cholesteryl acetate β-oxide (VI) by a reaction with potassium acetate and into 5 α-bromocholestan-3 β-ol-6-one acetate by oxidation with chromic acid in acetic acid.



(IV) (V) (VI)

It seemed to be of interest to study the allylic bromination with isocyanur bromide, since Ziegler and his coleagues[17] described the allylic chlorination of cyclohexene with isocyanur chloride. Reaction of cholesteryl acetate (IV), 4-cholesten-3-one and testosterone benzoate with isocyanur bromide in boiling carbon tetrachloride under a strong illumination, however, failed to produce brominated compounds but resulted in the recovery of the starting materials, except only in special case (See experimental part).

Experimental[18]

Isocyanur Bromide.—Ten grams of bromine and 60 ml. of distilled water were mixed and cooled in an ice-salt bath to 0°C. A solution of 2 g. of cyanuric acid (purity 92.5%[4]) and 3 g. of potassium hydroxide in 60 ml. of distilled water was added dropwise to the well-stirred mixture at 0~3°C, caution being taken not to cool down below 0°C. After the addition, the mixture was stirred for further twenty minutes. The solid was collected, washed thoroughly with ice water to neutral reaction, with purified acetone several times and twice with ether, (in order to remove the moisture quickly and stored in a sulfuric acid desiccator. Yield 5.3 g. or 75% of the theoretical.

Anal. Found: Br, 60.38% (micro Carius method; 92.2% of the theoretical); Br, 60.02% (Iodometry[4]; 91.8% of the theoretical). Calcd. for $C_3O_3N_3Br_3$: Br, 65.54%.

The purity was practically unchanged after stored in a sulfuric acid desiccator for three months in the dark. In other runs the percentage of bromine in the product ranged from 80% to 92%, doubtlessly owing to slight changes of washing procedures. When the precipitate was washed with dioxane and then with ether, nearly the same results were obtained. It took about ten days to dry the product completely over sulfuric acid and the purity became about 40~50%. When dried over phosphorous pentoxide under a reduced pressure (5 mmHg) overnight the purity also decreased to 55~60%.

Similar results were obtained when the reaction was carried out under illumination.

Cholestane-3 β, 5 α, 6 β-triol (II).—A suspension of 4.5 g. of cholesterol (I) in 200 ml. of acetone and 25 ml. of water was mixed with 3.3 g. (1.25 equiv.) of 55.1% isocyanur bromide and 2.5 ml. of acetic acid and the mixture was shaken occasionally at room temperature. Soon the mixture became yellow in color. In about one hour the whole solid went into solution and in 2.5 hours from the beginning a new solid appeared. After being kept over night, the suspension was filtered off and extracted with ether, and the extract was washed with water and alkali, dried and concentrated until crystals began to separate. After cooling with ice, the crystalline material was collected. Recrystallization from chloroform gave colorless needles melting at 237~238°C. Yield 1.0 g.

The identity was established by conversion into the diacetate[19], m.p. and mixed m.p. 165~166°C. The product did not produce a phenylhydrazone.

The ethereal mother liquor was evaporated under a reduced pressure and triturated with methanol to give 1.2 g. of a solid with m.p. 107~118°C*. Recrystallization from ethanol gave colorless needles, m.p. 121~122°C, which was identified as cholesterol dibromide by a mixed m.p. determination.

Cholestane-3 β, 5 β-diol-6-one (III).—To a solution of 251 mg. of compound II in 25 ml. of *tert*-butyl alcohol 0.5 ml. of pyridine, 0.5 ml. of water and 99.5 mg. of 83.8% isocyanur bromide were added successively. The mixture was warmed at 65~70°C for 30 minutes. After filtration the filtrate was diluted with ether, washed with a 10% bisulfite solution, aqueous alkali and water, and dried over anhydrous sodium sulfate. The solution was concentrated under a reduced pressure. Careful dilution with water gave needles, m.p. 230~233°C (decomp.), which was not depressed when admixed with an authentic sample[10]. Yield 228 mg.

The substance was further characterized by

16) Ueno obtained a small anount of 6β-bromocholestane-3β, 5α-diol 3-acetate from the mother liquor, but the present author did not attempt to isolate it.

17) K. Ziegler, A. Spath, E. Schauf, W. Schumann and E. Winklman, *Ann.*, **551**, 80 (1942).

18) All melting points are uncorrected. All rotations are measured in chloroform solution. Concentrations (*c*) are expressed in g. per 100 ml. of solution. The author is indebted to Mr. T. Iwama for microanalyses and to Mr. F. Mukawa for the supply of cyanuric acid.

19) V. A. Petrow, *J. Chem. Soc.*, **1937**, 1077.

* All of brominated steroids described in this paper melted with decomposition.

coversion into the phenylhydrazone[20], m.p. 163～164°C and into 3-monoacetate[10], m.p. 232～233°C.

Attempted Oxidation of Cholestane-3β, 5α, 6β-triol (II) with Isocyanur Bromide and Pyridine in Benzene.—A mixture of 0.25 g. of compound II, 20 ml. of benzene, 0.12 g. of 91.8% isocyanur bromide and 0.3 ml. of pyridine was heated under reflux for 15 minutes and treated as described above. The strating material, m.p. 215～225°C, was recovered. (0.19 g.) Recrystallized material melted at 236～238°C.

The identity was established by conversion into the diacetate[19], m.p. and mixed m.p. 165～166°C,

Hypobromous Acid Addition of Cholesteryl Acetate (IV).—(*a*) *Perchloric Acid-Isocyanur Bromide Method.* A solution of 1 g. of compound IV in 50 ml. of dioxane and 7 ml. of water was mixed with 0.6 g. of 84.5% isocyanur bromide and 0.3 ml. of 60% perchloric acid and the mixture was shaken occasionally at room temperature (19°C). After two hours the suspension was diluted with 25 ml. of a 10% sodium bisulfite solution and left to stand in a refrigerater (5°C) for one hour, and the solid was collected by filtration. The material was dried and extracted with a small volume of hot chloroform and the chloroform solution was diluted with petroleum ether. Crystals were collected. Needles, m.p. 160～165°C (0.68 g.). Recrystallization from chloroform-petroleum ether gave fine needles, m.p. 174～175°C, $[\alpha]_D^{21.5}$ −35 (c, 0.998). Yield 0.6 g. or 49% of the theoretical. The melting point was not depressed on admixture with authentic 5α-bromocholestane-3β, 6β-diol 3-monoacetate[15]. (Found: Br, 15.42%, Calcd. for $C_{29}H_{49}O_3Br$; Br 15.24%) Reported m.p. 177°C, $[\alpha]_D$ −37[21]; m.p. 175°C, $[\alpha]_D$ −33.8[12]; m.p. 177～178°C[15].

(*b*) *Perchloric Acid-N-Bromoacetamide Method.* A mixture of 1 g. of compound IV, 50 ml. of dioxane, 7 ml. of water, 0.45 g. of *N*-bromoacetamide and 0.3 ml. of 60% perchloric acid were treated in the same way as described above. Recrystallization from chloroform-petroleum ether gave needles, m.p. 158～161°C (0.9 g.). Further recrystallization yielded the pure substance, m.p. 174～175°C, $[\alpha]_D^{21}$ −38 (c, 1.02). Yield 0.75 g. or 61% of the theoretical. The melting point was not depressed on admixture with a specimen from method *a*.

(*c*) *Acetic Acid-Isocyanur Bromide Method.* A mixture of 2.5 g. of compound IV, 1.3 g. of 83.8% isocyanur bromide, 150 ml. of acetone, 10 ml. of water and 4 ml. of acetic acid was refluxed for ten minutes, poured into an excess of water, and extracted with ether. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated. The oily residue was dissolved in ethyl acetate and methanol was added to produce faint turbidity. Then a small volume of ether was added and the mixture was left to stand in a refrigerator (5°C) for 5 days. Crystals (m.p. 100～158°C) were collected and recrystallized from ether-methanol to produce long needles with m.p. 112～115°C (0.3 g.). The melting point was not depressed on admixture with authentic cholesteryl acetate dibromide.

The mother liquor from the above experiment was concentrated to produce crystals, which were mainly cholesteryl acetate dibromide (0.2 g.). The mother liquor from the above concentrate was again concentrated to produce crystals, which were collected and recrystallized from chloroform-petroleum ether to give needles, m.p. 174～175°C (0.2 g.). This substance did not depress the melting point of 5α-bromocholestane-3β, 6β-diol 3-monoacetate (V).

A solution of the mixture (0.75 g.) of the products from methods *a*, *b* and *c* in acetic acid (26 ml.) and water (7 drops) was oxidized with chromic acid (0.35 g.) to give 5α-bromocholestan-3β-ol-6-one acetate (0.6 g.), m. p. 165～166°C. (Found: Br, 15.18%. Calcd. for $C_{29}H_{47}O_3Br$: Br, 15.26%). Reported m.p. 164～166°C[12], 164～165°C[15], 163°C[22].

Cholesteryl Acetate β-Oxide (VI).—To a suspension of 212 mg. of compound V in 7 ml. of ethanol was added 0.4 g. of sodium acetate (crystalline) and the mixture was refluxed for 1.5 hours. Soon the solid went wholly into solution and a new solid appeared. After cooling, water was added and the solid was collected by filtration. The crystals, after recrystallized from aqueous methanol, gave a negative Beilsterin test, and melted at 111～112°C. Needles (189 mg.). The melting point was not depressed when admixed with authentic cholesteryl acetatate β-oxide[24] (VI). Reported m.p. 111～113°C; $[\alpha]_D$ −1[23], 113°C[24].

The product (21.2 mg.) in chloroform (0.5 ml.) was treated with 4 N hydrogen bromide in acetic acid (0.5 ml.) for 10 minutes at room temperature to give needles with m.p. 172～174°C when recrystallized from chloroform-petroleum ether. The melting point was not depressed on admixture with 5α-bromocholestane-3β, 6β-diol 3-monoacetate (V).

Attempted Allylic Bromination of Testosterone Benzoate.—(*a*) *With Isocyanur Bromide washed with Acetone as Described Above.* A mixture of 0.5 g. of testosterone benzonate and 0.25 g. of 91.8% isocyanur bromide in 10 ml. of carbon tetrachloride was refluxed for 30 minutes with exposure to strong light. The starting material was recovered in nearly quantitative yield. Under nearly the same conditions, 4-cholesten-3-one and cholesteryl acetate could not be brominated.

(*b*) *With Isocyanur Bromide not washed with Acetone but left to stand in a Sulfuric Acid Desiccator Overnight.* To a solution of 1 g. of testosterone benzoate in 20 ml. of carbon tetrachloride was added 1.2 g. of the isocyanur bro-

20) R. H. Pickard and J. Yates, ibid., **93**. 1678 (1908).
21) D. R. Jones and C. W. Shoppee, ibid., **1954**, 4224.
22) I. M. Heilbron, E. R. H. Jones and F. S. Spring, ibid., **1937**, 801.
23) P. A. Plattner et al., *Helv. chim. Acta*, **27**, 513 (1944).
24) S. Mori, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **64**, 981 (1943).

mide and the mixture was refluxed for 30 minutes with exposure to strong light. The solid was removed by filtration and the solution was evaporated to dryness. Recrystallization from acetone-methanol gave needles with m.p. 150~152°C (0.55 g.). After recrystallization from ethyl acetate-methanol, the analytical sample showed m.p. 178°C, $[\alpha]_D^{19}+86$ (c, 0.370), λ_{max} 232 mμ (ε 23,800).

Anal. Found: Br, 17.29%. Calcd. for $C_{26}H_{31}O_3Br$: Br, 16.96%.

Ruzicka et al.[25] reported m.p. 176~177°C for 6ξ-bromotestosterone benzoate. The product may be 6ζ-bromotestosterone benzoate, since the same substance was obtained when testoserone benzoate was brominated with *N*-bromosuccinimide as described below and it is known that reactions of 4-ene-3-ketosteroids and *N*-bromosuccinimide give corresponding 6ξ-bromo-4-ene-3-ketosteroids[26]. But the abnormal shift of the ultraviolet absorption maximum to the shorter wavelength can not be explained[27].

(*c*) *With N-Bromosuccinimide.* One half gram of testosterone benzoate was treated with 0.25 g. of *N*-bromosuccinimide in refluxing 10 ml. of carbon tetrachloride for 30 minutes under the ordinary light. After evaporation under a reduced pressure, the residue was triturated with *n*-hexane to give a colorless solid (0.45 g.), m.p. 143~144°C. The melting point was raised to 177~177.5°C when recrystallized from ethyl acetate-methanol and was not dedressed on admixture with the sample obtained above.

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25) R. Ruzicka et al., *Helv. chim. Acta*, **20**, 328 (1937).
26) C. Djerassi et al., *J. Am. Chem. Soc.*, **72**, 4534 (1950); F. Sondheimer et al., ibid., **75**, 4712, 5932 (1953); B. Camerino et al., *Gazz. chim. ital.*, **84**, 301 (1954); *Chem. Abst.*, **49**, 14787b (1955); V. R. Mattox et al., *J. Biol. Chem.*, **197**, 261 (1952).
27) Cf. K. Morita, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **78**, 1581 (1957).