[Chem. Pharm. Bull. 14(6) 613~618 (1966)] UDC 547.918.07 : 615.711.5

87. Daisuke Satoh, Mieko Horie, and Junko Morita : Studies on Digitalis Glycosides. XXIV.*1 Ring-opening Reaction of 14,15-Epoxide with Thiocyanic Acid, and Preparation of 3β-Hydroxy-14β,15β-epithio-5β-card-20(22)-enolide.*2

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Ring-opening reaction of steroidal epoxide with thiocyanic acid was initiated by Mosettig[1] and Takeda[2] at about the same time, and steroids having episulfide ring in many positions, such as 2,3-,[3~5] 3,4-,[6] 5,6-,[6~8] 11,12-,[9~11] and 16,17-positions,[11] have been prepared from the corresponding epoxides.

On the other hand, some epoxycardenolides, such as 8,14-,[12] 11,12-,[13] 14,15-[14~18] and 16,17-epoxides,[19] were found in nature or synthesized in recent years, but the episulfide of cardenolide has never been known. This paper is concerned with thiocyanic acid opening of 14,15-epoxides and the preparation of 14β,15β-episulfide of cardenolide.

When 3β-acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ib) was treated with a chloroform or an ether solution of thiocyanic acid, two products were detected by thin-layer chromatography. The reaction mixture was chromatographed on silica gel affording a product, $C_{26}H_{35}O_5NS$, m.p. 215~218°, together with the starting material (Ib). The infrared spectrum of this product exhibited a strong absorption band at 2167 cm^{-1} corresponding to an isothiocyanato group[20] besides those due to a hydroxyl group and a butenolide ring. From these results the product seemed to belong to an isothiocyanatohydrine of cardenolide. Since the direction of opening of 14α,15α-epoxide ring with thiocyanic acid was assumed to be analogous to that with hydrogen chloride,[15] the structure of 3β-acetoxy-14β-isothiocyanato-15α-hydroxy-5β-card-20(22)-enolide (III) was assigned to the product. This assignment was confirmed as described below.

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*1 Part XXIII. T. Wada : This Bulletin, **13**, 312 (1965).

*2 A part of this work has been reported in brief in the review entitled "Studies on the Constituents of *Digitalis purpurea* L. Leaves" published in Ann. Rept. Shionogi Res. Lab., **14**, 14 (1964).

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The facts that the expected thiocyanatohydrine (II) could not be isolated in the process described above and, moreover, the epoxide (Ib) which was not detected in the reaction mixture was separated after chromatography on silica gel, suggested regeneration of the epoxide (Ib) from II by ring closure during the separation procedure. In order to avoid this disadvantage, the crude reaction product was mesylated directly and chromatographed on silica gel to yield three crystalline products (A,B,C).

Product A, $C_{27}H_{37}O_7NS_2$, m.p. 155~158°, exhibited a weak absorption band at 2160 cm^{-1} due to thiocyanato group[1] and a strong absorption band at 1170 cm^{-1} ascribable to mesyloxy group besides those of butenolide ring in the infrared spectrum. This finding showed that product A belong to a thiocyanatohydrine, thus having the structure 3β-acetoxy-14β-thiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (IVb). Hydrolysis of IVb with dilute hydrochloric acid gave 3β-hydroxy compound (IVa), $C_{25}H_{35}O_6NS_2$, m.p. 152~154°. Product B, $C_{27}H_{37}O_7NS_2$, m.p. 195~197°, is an isomer of product A and its infrared spectrum exhibited strong absorption bands at 2040 cm^{-1} and 1169 cm^{-1} ascribable to isothiocyanato group and mesyloxy group, respectively, besides those of butenolide ring. These data corresponded to the structure 3β-acetoxy-14β-isothiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (V). As it is well known that thiocyanic acid exists in a tautomeric form indicated as $N\equiv C\text{-}SH \rightleftarrows HN=C=S$, the formation of both thiocyanate (IV) and isothiocyanate (V) in the above mentioned reaction could be rationalized. Mosettig[21] and Komeno[7] also recognized formation of isothiocyanates in the ring-opening reaction of epoxides with thiocyanic acid on the basis of infrared data, but they did not isolate their isothiocyanates in pure state. Product C, $C_{26}H_{33}O_4NS$, m.p. 199~202°, exhibited an absorption band at 2166 cm^{-1} due to thiocyanato group besides those of butenolide ring, but no absorption band of hydroxyl and mesyloxy groups in the infrared spectrum. A positive tetranitromethane test showed the presence of a double bond in its molecule and the ultraviolet absorption maximum at 215 mμ indicated no conjugation of the double bond with the butenolide ring. From these data it seemed to be most reasonable to assign the formula 3β-acetoxy-14β-thiocyanato-5β-card-15,20(22)-dienolide (VI) to product C. This compound must be formed by elimination of a methane sulfonic acid from product A.

As cardenolides are not so stable to strong alkali, alkali hydrogen carbonate was used for the episulfide ring closure of thiocyanatohydrine mesylate. Thus 3β-acetoxy-14β-thiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (IVb) was treated with 1~2% potassium hydrogen carbonate in aqueous methanol at room temperature for three days, and the reaction mixture was shown to contain two products besides the intact starting material by thin-layer chromatography. The crude product was separated by preparative thin-layer chromatopraphy. The major product $C_{25}H_{34}O_4S$, m.p. 195~198°, exhibited absorption band of a butenolide ring and no absorption bands due to thiocyanato and mesyloxy groups in the infrared spectrum in spite of having one atom of sulfur in its molecule. From these facts this product was thought to be 3β-acetoxy-14β,15β-epithio-5β-card-20(22)-enolide (VIIb). A signal at 6.57τ (J=2.5 c.p.s.) due to a proton at C-15 position in the nuclear magnetic resonance supported this formula.[22] The minor product was found to be 3β-acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ib).

When 3β-hydroxythiocyanatohydrine mesylate (IVa) was submitted to the similar reaction to that described above, 3β-hydroxy-14β,15β-epithio-5β-card-20(22)-enolide (VIIa) $C_{23}H_{32}O_3S$, m.p. 173~176°, was obtained together with 3β-hydroxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ia). Acetylation of VIIa afforded the acetate (VIIb).

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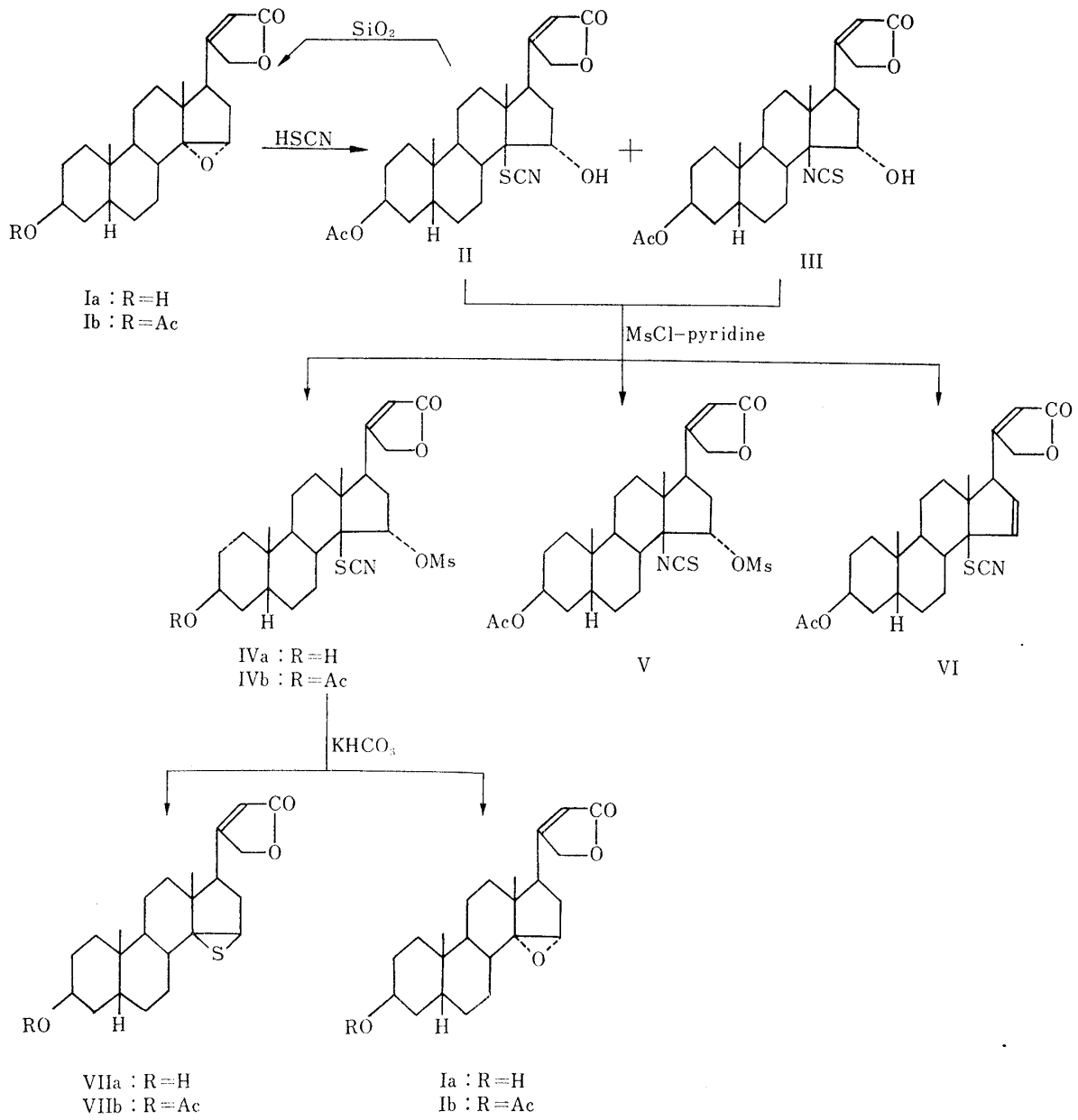

Chart 1.

The ring-opening of 3β-acetoxy-14β,15β-epoxy-5β-card-20(22)-enolide (Ⅷ), on the other hand, did not proceed so smoothly as that of the 14α,15α-epoxide (Ib). In the reaction mixture of Ⅷ with a chloroform solution of thiocyanic acid the starting material was detected besides two products by thin-layer chromatography even after four days. This inertness for ring-opening reaction is probably attributable to a steric hindrance caused by C/D-*cis* juncture. From the crude reaction mixture two products were obtained in crystalline form together with the intact starting material by preparative thin-layer chromatography. The first product $C_{26}H_{35}O_5NS$, m.p. 190~195°, exhibited absorption bands corresponding to thiocyanatohydrine at 3462 and 2160 cm^{-1} besides those due to a butenolide ring in the infrared spectrum, and furthermore, it did not undergo any acetylation. From these results the first product was considered to be 3β-acetoxy-14β-hydroxy-15α-thiocyanato-5β-card-20(22)-enolide (Ⅸ). It regenerated Ⅷ in contact with silica gel for some time. The second product $C_{25}H_{34}O_5$, m.p. 225~230°,

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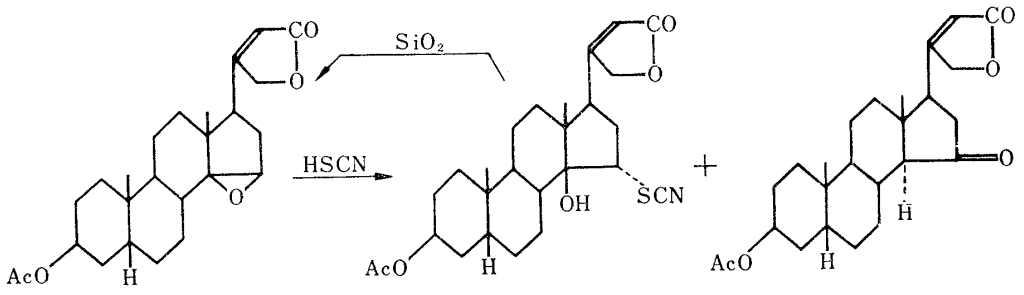
was identical with an authentic sample of 3β-acetoxy-15-oxo-5β,14α-card-20(22)-enolide (X) with respect to mixed melting point and infrared spectrum. Formation of X from Ⅷ can be explained by a *cis*-hydride shift of the 14β,15β-epoxide ring.[15,23] In the ring-opening reaction of Ⅷ an insignificant formation of isothiocyanatohydrine was indicated by the infrared spectrum of the crude reaction product.



VIII IX X

Chart 2.

Experimental*4

Preparation of Thiocyanic Acid-Chloroform Solution——A slurry of 4.8 g. of powdered KSCN in 20 ml. of $CHCl_3$ was triturated with 7.5 g. of $KHSO_4$ in a mortar for 5 min. After HSCN-$CHCl_3$ solution was decanted, an additional 6 ml. of $CHCl_3$ was added to the solid mixture. The mixture was triturated for an additional 5 min. and then filtered through cotton. The $CHCl_3$ solution was combined and this solution showed to contain 64 mg./ml. of HSCN in titration with 0.1 *N* NaOH solution.

Preparation of Thiocyanic Acid-Ether Solution——In a 50 ml. dropping funnel 1.8 g. of KSCN was dissolved in a small amount of H_2O and shaved ice, and 8 ml. of ether was added. Under shaking the mixture, 2.7 g. of H_3PO_4 was added in small portions. The ether layer was washed with a small amount of H_2O, dried over Na_2SO_4 and filtered through cotton. The ether solution showed to contain 67 mg./ml. of HSCN.

3β-Acetoxy-14β-isothiocyanato-15α-hydroxy-5β-card-20(22)-enolide (III)——To 4 ml. of HSCN-$CHCl_3$ solution (64 mg./ml.), 200 mg. of 3β-acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ib) was added and the solution was allowed to stand at room temperature, protected from light, for 5 hr.: no presence of Ib was indicated by thin-layer chromatography (TLC). (SiO_2, toluene-AcOEt=1 : 1). The reaction mixture was diluted with $CHCl_3$, washed with 10% $KHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated under reduced pressure. The residue 235 mg. was separated in two products by column chromatography using 12 g. of SiO_2 as an adsorbent and toluene-AcOEt=1 : 1 as an eluent.

i) More polar fraction (70 mg.) was crystallized from MeOH to 38 mg. of needles of 3β-acetoxy-14β-isothiocyanato-15α-hydroxy-5β-card-20(22)-enolide (Ⅲ), m.p. 215~218°. *Anal.* Calcd. for $C_{26}H_{35}O_5NS$: C, 65.93; H, 7.45; N, 2.96; S, 6.77. Found: C, 65.86; H, 7.39; N, 2.87; S, 6.62. IR ν_{max}^{Nujol} cm^{-1}: 3407 (OH), 2167, 2067 (-NCS), 1784, 1725, 1627 (butenolide).

ii) Less polar fraction (65 mg.) was crystallized from MeOH to 35 mg. of crystals of 3β-acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ib), m.p. 196~203°. Mixed melting point and IR spectrum established the identity with an authentic sample.

3β-Acetoxy-14β-thiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (IVb) and 3β-Acetoxy-14β-isothiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (V)——1) Fission in HSCN-$CHCl_3$ solution: To 42 ml. of HSCN-$CHCl_3$ solution (64 mg./ml.), 1.9 g. of 3β-acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ib) was added and the solution was allowed to stand at room temperature for 3 hr. and the reaction solution was treated as above mentioned. The crude mixture of fission products was dissolved in 21 ml. of pyridine, and 2.1 ml. of MsCl was added under cooling below 0°, and the mixture was kept in a refrigerator. After 3 days the reaction was observed to be finished and formation of two main products besides a few by-products was indicated by TLC (SiO_2, toluene-AcOEt=3 : 1). The solution was poured into ice-water and extracted with $CHCl_3$, washed with H_2O, dried over Na_2SO_4 and evaporated under reduced pressure. The crude mixture of mesylates (2.426 g.) was separated into three fractions by column chromatography using 120 g. of SiO_2, and mixtures of $CHCl_3$ and benzene.

*4 All melting points are uncorrected.

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i) Most polar fraction (A, 1.201 g. eluted with $CHCl_3$–benzene=3 : 1 and 5 : 1) was crystallized from MeOH to 855 mg. of needles of 3β-acetoxy-14β-thiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (Ⅳb), m.p. 155～158°. $[\alpha]_D^{24}$ + 22.1° (c=1.023, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{37}O_7NS_2$: C, 58.78; H, 6.76; N, 2.54; S, 11.62. Found : C, 58.51; H, 7.01; N, 2.61; S, 11.50. UV λ_{max}^{EtOH} mμ (ε) : 213.5 (15,561). IR ν_{max}^{Nujol} cm^{-1} : 2160 (SCN), 1789, 1743, 1622 (butenolide), 1715 (Ac), 1170 (OMs).

ii) More polar fraction (B, 553 mg. eluted with $CHCl_3$–benzene=2 : 1) was crystallized from MeOH to 364 mg. of needles of 3β-acetoxy-14β-isothiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (Ⅴ), m.p. 195～197°. $[\alpha]_D^{24}$ + 6.3° (c=1.038, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{37}O_7NS_2$: C, 58.78; H, 6.76; N, 2.54; S, 11.62. Found : C, 58.81; H, 6.82; N, 2.34; S, 11.28. UV λ_{max}^{EtOH} mμ (ε): 216 (12,951). IR ν_{max}^{Nujol} cm^{-1}: 2040 (NCS), 1782, 1744, 1632 (butenolide), 1719 (Ac), 1169 (OMs).

iii) Less polar fraction (C, 110 mg. eluted with $CHCl_3$–benzene=1 : 1) was crystallized from MeOH to 55 mg. of needles of 3β-acetoxy-14β-thiocyanato-5β-card-15,20(22)-dienolide (Ⅵ), m.p. 199～202°. *Anal.* Calcd. for $C_{26}H_{33}O_4NS$: C, 68.52; H, 7.30; N, 3.07; S, 7.04. Found : C, 68.74; H, 7.40; N, 3.11; S, 7.14. UV λ_{max}^{EtOH} mμ (ε) : 215 (25,000). IR ν_{max}^{Nujol} cm^{-1} : 2166 (SCN), 1787, 1752, 1640 (butenolide), 1732 (Ac).

2) Fission in HSCN–ether solution : To 7 ml. of the above-mentioned HSCN–ether solution, 300 mg. of Ib was added, the mixture was agitated at room temperature for some time and allowed to stand for 3 days until no presence of Ib was observed by TLC (SiO_2, toluene–AcOEt=3 : 1). The reaction mixture was washed with 10% $NaHCO_3$ and H_2O, dried over Na_2SO_4, evaporated to dryness.

The crude product (305 mg.) was mesylated in 3 ml. of pyridine with 0.3 ml. of MsCl below 0°. After keeping in a refrigerator for 3 days, the reaction mixture was treated in the usual manner. The crude product showed the presence of two main products together with a few by-products on TLC (SiO_2, toluene–AcOEt=3 : 1) analogously in the fission reaction in HSCN–$CHCl_3$ as described above. The crude mixture was separated by a silica gel column into 68 mg. of Ⅳb and 60 mg. of Ⅴ.

3β-Hydroxy-14β-thiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (IVa)——In 20 ml. of 1% HCl (MeOH–$CHCl_3$=4 : 1) 100 mg. of 3β-acetoxy-14β-thiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (Ⅳb) was dissolved and the solution was set aside overnight at room temperature. After dilution with H_2O, neutralized with dil. Na_2CO_3, concentrated under reduced pressure, the precipitate formed was extracted with $CHCl_3$, washed with H_2O, dried over Na_2SO_4, and $CHCl_3$ was evaporated to dryness. The crude product was crystallized from MeOH to 62 mg. of needles of 3β-hydroxy-14β-thiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (Ⅳa), m.p. 152～154°. *Anal.* Calcd. for $C_{25}H_{35}O_6NS_2$: C, 58.91; H, 6.92; N, 2.75; S, 12.58. Found : C, 58.85; H, 7.02; N, 2.62; S, 12.39. IR ν_{max}^{Nujol} cm^{-1} : 3440, 3380 (OH), 2160 (SCN), 1795, 1735, 1625 (butenolide),1170 (OMs).

Acetylation of 15 mg. of Ⅳa with 0.15 ml. of pyridine and Ac_2O in the usual manner gave needles of Ⅳb, m.p. 154～157°.

3β-Acetoxy-14β,15β-epithio-5β-card-20(22)-enolide (VIIb)——To 180 ml. of 1% $KHCO_3$ (MeOH–H_2O= 4 : 1) 200 mg. of 3β-acetoxy-14β-thiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (Ⅳb) was added and the solution was allowed to stand for 3 days at room temperature. While the starting material was detected even at this time, the formation of by-product was observed by TLC (SiO_2, $CHCl_3$–acetone=40 : 1). The reaction mixture was neutralized with dil. HCl, concentrated under reduced pressure, extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. As TLC of the residue showed the existence of three main components, the residue was separated into three fractions by preparative TLC (SiO_2, $CHCl_3$–acetone=40 : 1).

i) Less polar fraction (40 mg.) was crystallized from MeOH to 29 mg. of prisms of 3β-acetoxy-14β,15β-epithio-5β-card-20(22)-enolide (Ⅶb), m.p. 195～198°. $[\alpha]_D^{25}$ +52.2 (c=1.031, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{34}O_4S$: C, 69.73; H, 7.96; S, 7.45. Found: C, 69.61; H, 8.04; S, 7.43. UV λ_{max}^{EtOH} mμ (ε) : 213.5 (14,910). IR ν_{max}^{Nujol} cm^{-1} : 1784, 1745, 1627 (butenolide), 1745, 1245 (Ac).

ii) More polar fraction (20 mg.) was crystallized from MeOH to 11 mg. of 3β-acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ib), m.p. 199～206°. Mixed melting point and IR spectrum showed the identity with an authentic sample.

iii) Most polar fraction (50 mg.) was shown to be the starting material recovered intact by mixed fusion and IR spectrum.

3β-Hydroxy-14β,15β-epithio-5β-card-20(22)-enolide (VIIa)——In 250 ml. of 2% $KHCO_3$ (MeOH–H_2O= 4:1) 500 mg. of 3β-hydroxy-14β-thiocyanato-15α-mesyloxy-5β-card-20(22)-enolide (Ⅳa) was dissolved and the solution was allowed to stand at room temperature for 3 days. The crude product obtained by the usual treatment was separated by preparative TLC (SiO_2, $CHCl_3$–acetone=5 : 1) into three fractions.

i) Less polar fraction (105 mg.) was crystallized from AcOEt to 56 mg. of needles of 3β-hydroxy-14β,15β-epithio-5β-card-20(22)-enolide (Ⅶa), m.p. 173～176°. $[\alpha]_D^{23.5}$ +62.6° (c=0.566, MeOH). *Anal.* Calcd. for $C_{23}H_{32}O_3S$: C, 71.09, H, 8.30; S, 8.25. Found : C, 70.95; H, 8.41; S, 8.07. IR ν_{max}^{Nujol} cm^{-1} : 3320 (OH), 1783, 1748, 1620 (butenolide).

Acetylation of this compound by the usual method gave Ⅶb, m.p. 192～195°.

ii) More polar fraction (50 mg.) was crystallized from MeOH to give 23 mg. of 3β-hydroxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ia), m.p. 241～245°.

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iii) Most polar fraction (135 mg.) was identified with the starting material by mixed melting point and IR spectrum.

3β-Acetoxy-14β-hydroxy-15α-thiocyanato-5β-card-20(22)-enolide (IX)——To 10 ml. of HSCN-$CHCl_3$ solution (34 mg./ml.) 224 mg. of 3β-acetoxy-14β,15β-epoxy-5β-card-20(22)-enolide (Ⅷ) was added and the solution was allowed to stand at room temperature, protected from light, for 4 days. The reaction mixture was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was separated directly into three fractions by preparative TLC (SiO_2, acetone-$CHCl_3$=1 : 5 and toluene-AcOEt=1 : 1) in order to avoid regeneration of the epoxide (Ⅷ) from thiocyanatohydrine.

i) The first fraction (58 mg.) was the starting material (Ⅷ) recovered intact.

ii) The second fraction (109 mg.) was crystallized to 56 mg. of needles of 3β-acetoxy-14β-hydroxy-15α-thiocyanato-5β-card-20(22)-enolide (Ⅸ), m.p. 190~195°. $[\alpha]_D^{24.5}$ +76.5° (c=0.518, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{35}O_5NS \cdot \frac{1}{2}H_2O$: C, 64.70; H, 7.52; N, 2.90; S, 6.74. Found : C, 64.75; H, 7.40; N, 3.20; S, 6.78. UV λ_{max}^{EtOH} mμ (ε): 215 (17,300). IR ν_{max}^{Nujol} cm^{-1}: 3462(OH), 2160(SCN), 1786, 1746, 1625 (butenolide), 1694 (Ac).

iii) The third fraction (58 mg.) was crystallized to 15 mg. of needles of 3β-acetoxy-15-oxo-5β,14α-card-20(22)-enolide (X), m.p. 225~230°. Mixed melting point and IR spectrum established the identity with an authentic sample of X.

The authors express their deep gratitude to Dr. K. Takeda, the Director of this Laboratory, for his unfailing guidance, and to Dr. T. Komeno for his valuable advices. They are grateful to the members of the Analysis Room for elemental analytical data, and to the members of the Section of Physical Chemistry for measurements of optical data and nuclear magnetic resonance spectra.

Summary

Ring-opening reaction of 3β-acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ib) with thiocyanic acid proceeded smoothly to afford 3β-acetoxy-14β-thiocyanato-15α-hydroxy-5β-card-20(22)-enolide (Ⅱ) and 3β-acetoxy-14β-isothiocyanato-15α-hydroxy-5β-card-20(22)-enolide (Ⅲ). On the contrary, the reaction with 3β-acetoxy-14β,15β-epoxy-5β-card-20(22)-enolide (Ⅷ) did not proceed so smoothly as a result of steric hindrance, giving 3β-acetoxy-14β-hydroxy-15α-thiocyanato-5β-card-20(22)-enolide (Ⅸ) together with 3β-acetoxy-15-oxo-5β,14α-card-20(22)-enolide (Ⅹ). Ring closure of the mesylate of Ⅱ with a weak alkali resulted in the formation of 3β-acetoxy-14β,15β-epithio-5β-card-20(22)-enolide (Ⅷb) and 3β-acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide (Ib).

(Received October 25, 1965)

[Chem. Pharm. Bull. 14(6) 618~621 (1966)]

UDC 543.544.25 : 547.564.4.08

88. Satoshi Kawai*1, Toshiharu Nagatsu*2, Toshio Imanari, and Zenzo Tamura*1 : Gas Chromatography of Catecholamines and Related Compounds.

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A number of physiologically important amines have been successfully analyzed by gas chromatography. But effective methods for the gas chromatographic analysis of catecholamines are still lacking. This is largely because these amines are strongly polar, nonvolatile, rather insoluble in most organic solvents and extremely unstable.

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