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

(Shionogi Research Laboratory, Shionogi & Co., Ltd.*³)

Ring-opening reaction of steroidal epoxide with thiocyanic acid was initiated by Mosettig¹⁾ and Takeda²⁾ 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 14 β ,15 β -episulfide of cardenolide.

When 3 β -acetoxy-14 α ,15 α -epoxy-5 β -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, C₂₆H₃₆O₆NS, 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 group²⁰⁾ 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 14 α ,15 α -epoxide ring with thiocyanic acid was assumed to be analogous to that with hydrogen chloride,¹⁵⁾ the structure of 3 β -acetoxy-14 β -isothiocyanato-15 α -hydroxy-5 β -card-20(22)-enolide (III) was assigned to the product. This assignment was confirmed as described below.

*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).

*³ Fukushima-ku, Osaka (佐藤大助, 堀江美恵子, 森田淳子).

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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 group¹⁾ 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 3 β -acetoxy-14 β -thiocyanato-15 α -mesyloxy-5 β -card-20(22)-enolide (Nb). Hydrolysis of Nb with dilute hydrochloric acid gave 3 β -hydroxy compound (Na), $C_{25}H_{35}O_6NS_2$, 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 3 β -acetoxy-14 β -isothiocyanato-15 α -mesyloxy-5 β -card-20(22)-enolide (V). As it is well known that thiocyanic acid exists in a tautomeric form indicated as $N\equiv C-SH \rightleftharpoons HN=C=S$, the formation of both thiocyanate (IV) and isothiocyanate (V) in the above mentioned reaction could be rationalized. Mosettig²¹⁾ and Komeno⁷⁾ 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 m μ indicated no conjugation of the double bond with the butenolide ring. From these data it seemed to be most reasonable to assign the formula 3 β -acetoxy-14 β -thiocyanato-5 β -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 3 β -acetoxy-14 β -thiocyanato-15 α -mesyloxy-5 β -card-20(22)-enolide (Nb) 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 chromatography. 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 3 β -acetoxy-14 β ,15 β -epithio-5 β -card-20(22)-enolide (VIIb). A signal at 6.57 τ ($J=2.5$ 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 3 β -acetoxy-14 α ,15 α -epoxy-5 β -card-20(22)-enolide (Ib).

When 3 β -hydroxythiocyanatohydrine mesylate (Na) was submitted to the similar reaction to that described above, 3 β -hydroxy-14 β ,15 β -epithio-5 β -card-20(22)-enolide (VIIa) $C_{23}H_{32}O_3S$, m.p. 173~176°, was obtained together with 3 β -hydroxy-14 α ,15 α -epoxy-5 β -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 C₂₈H₃₈O₅NS, 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 C₂₅H₃₄O₅, m.p. 225~230°,

was identical with an authentic sample of 3 β -acetoxy-15-oxo-5 β ,14 α -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 14 β ,15 β -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.

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.

3 β -Acetoxy-14 β -isothiocyanato-15 α -hydroxy-5 β -card-20(22)-enolide (III)—To 4 ml. of HSCN-CHCl_3 solution (64 mg./ml.), 200 mg. of 3 β -acetoxy-14 α ,15 α -epoxy-5 β -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 3 β -acetoxy-14 β -isothiocyanato-15 α -hydroxy-5 β -card-20(22)-enolide (III), m.p. 215~218°. *Anal.* Calcd. for $\text{C}_{26}\text{H}_{35}\text{O}_5\text{NS}$: 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 $\nu_{\text{max}}^{\text{Nujol}}$ 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 3 β -acetoxy-14 α ,15 α -epoxy-5 β -card-20(22)-enolide (Ib), m.p. 196~203°. Mixed melting point and IR spectrum established the identity with an authentic sample.

3 β -Acetoxy-14 β -thiocyanato-15 α -mesyloxy-5 β -card-20(22)-enolide (IVb) and 3 β -Acetoxy-14 β -isothiocyanato-15 α -mesyloxy-5 β -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 3 β -acetoxy-14 α ,15 α -epoxy-5 β -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 3 β -acetoxy-14 β -thiocyanato-15 α -mesyloxy-5 β -card-20(22)-enolide (IVb), m.p. 155~158°. $[\alpha]_D^{25} + 22.1^\circ$ ($c=1.023$, CHCl_3). *Anal.* Calcd. for $\text{C}_{27}\text{H}_{37}\text{O}_7\text{NS}_2$: 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 $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ): 213.5 (15,561). IR $\nu_{\text{max}}^{\text{Nujol}}$ 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 3 β -acetoxy-14 β -isothiocyanato-15 α -mesyloxy-5 β -card-20(22)-enolide (V), m.p. 195~197°. $[\alpha]_D^{25} + 6.3^\circ$ ($c=1.038$, CHCl_3). *Anal.* Calcd. for $\text{C}_{27}\text{H}_{37}\text{O}_7\text{NS}_2$: 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 $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ): 216 (12,951). IR $\nu_{\text{max}}^{\text{Nujol}}$ 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 3 β -acetoxy-14 β -thiocyanato-5 β -card-15,20(22)-dienolide (VI), m.p. 199~202°. *Anal.* Calcd. for $\text{C}_{26}\text{H}_{33}\text{O}_4\text{NS}$: 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 $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ): 215 (25,000). IR $\nu_{\text{max}}^{\text{Nujol}}$ 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.

3 β -Hydroxy-14 β -thiocyanato-15 α -mesyloxy-5 β -card-20(22)-enolide (IVa)—In 20 ml. of 1% HCl (MeOH- CHCl_3 =4:1) 100 mg. of 3 β -acetoxy-14 β -thiocyanato-15 α -mesyloxy-5 β -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 3 β -hydroxy-14 β -thiocyanato-15 α -mesyloxy-5 β -card-20(22)-enolide (IVa), m.p. 152~154°. *Anal.* Calcd. for $\text{C}_{26}\text{H}_{35}\text{O}_6\text{NS}_2$: 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 $\nu_{\text{max}}^{\text{Nujol}}$ 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°.

3 β -Acetoxy-14 β ,15 β -epithio-5 β -card-20(22)-enolide (VIIb)—To 180 ml. of 1% KHCO_3 (MeOH- H_2O =4:1) 200 mg. of 3 β -acetoxy-14 β -thiocyanato-15 α -mesyloxy-5 β -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 3 β -acetoxy-14 β ,15 β -epithio-5 β -card-20(22)-enolide (VIIb), m.p. 195~198°. $[\alpha]_D^{25} + 52.2^\circ$ ($c=1.031$, CHCl_3). *Anal.* Calcd. for $\text{C}_{25}\text{H}_{34}\text{O}_4\text{S}$: C, 69.73; H, 7.96; S, 7.45. Found: C, 69.61; H, 8.04; S, 7.43. UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ): 213.5 (14,910). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 1784, 1745, 1627 (butenolide), 1745, 1245 (Ac).

ii) More polar fraction (20 mg.) was crystallized from MeOH to 11 mg. of 3 β -acetoxy-14 α ,15 α -epoxy-5 β -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.

3 β -Hydroxy-14 β ,15 β -epithio-5 β -card-20(22)-enolide (VIIa)—In 250 ml. of 2% KHCO_3 (MeOH- H_2O =4:1) 500 mg. of 3 β -hydroxy-14 β -thiocyanato-15 α -mesyloxy-5 β -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 3 β -hydroxy-14 β ,15 β -epithio-5 β -card-20(22)-enolide (VIIa), m.p. 173~176°. $[\alpha]_D^{25} + 62.6^\circ$ ($c=0.566$, MeOH). *Anal.* Calcd. for $\text{C}_{23}\text{H}_{32}\text{O}_3\text{S}$: C, 71.09; H, 8.30; S, 8.25. Found: C, 70.95; H, 8.41; S, 8.07. IR $\nu_{\text{max}}^{\text{Nujol}}$ 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 3 β -hydroxy-14 α ,15 α -epoxy-5 β -card-20(22)-enolide (Ia), m.p. 241~245°.

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

3 β -Acetoxy-14 β -hydroxy-15 α -thiocyanato-5 β -card-20(22)-enolide (IX)—To 10 ml. of HSCN-CHCl₃ solution (34 mg./ml.) 224 mg. of 3 β -acetoxy-14 β ,15 β -epoxy-5 β -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 H₂O, dried over Na₂SO₄ and evaporated *in vacuo*. The crude product was separated directly into three fractions by preparative TLC (SiO₂, acetone-CHCl₃=1:5 and toluene-AcOEt=1:1) in order to avoid regeneration of the epoxide (VIII) from thiocyanatohydride.

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 3 β -acetoxy-14 β -hydroxy-15 α -thiocyanato-5 β -card-20(22)-enolide (IX), m.p. 190~195°. $[\alpha]_D^{25} +76.5^\circ$ (c=0.518, CHCl₃). *Anal.* Calcd. for C₂₆H₃₅O₅NS·½H₂O: 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} m μ (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 3 β -acetoxy-15-oxo-5 β ,14 α -card-20(22)-enolide (X), m.p. 225~230°. Mixed melting point and IR spectrum established the identity with an authentic sample of X.

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Summary

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

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

(Faculty of Pharmaceutical Sciences, University of Tokyo*¹
and Department of Biochemistry, School of
Dentistry, Aichi-Gakuin University*²)

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.

*¹ Hongo, Tokyo (河合 聡, 今成登志男, 田村善蔵).

*² Chikusaku, Nagoya (永津俊治).