Therefore, three peaks corresponding to authentic cyclohexylamine, cyclohexanone, and cyclohexanol were detected from the sample solutions (C) and (D) as shown in Fig. 2 and 3.

Metabolism of CHS-Na in Rat Liver *in Vitro*

In order to study further on the CHS–Na metabolites detected in the urine from rabbit and rat, an experiment utilizing rat liver homogenate was carried out.

The rat liver homogenate containing 100 mg of CHS–Na was incubated on an incubator at a shake rate of 120 per minute and a temperature of 37° for 90 minutes. Then, the sample solutions (E and F) were prepared from the supernatant which was obtained by centrifuging the homogenate for 20 minutes at 9000 rpm (see Fig. 1), and were submitted to gas chromatography. As shown in Fig. 4, the gas chromatograms of the above sample solutions indicated the presence of cyclohexylamine, cyclohexanone, and cyclohexanol as metabolic products of CHS–Na, though these metabolites were a very small amount.

Metabolism of Cyclohexylamine in Rabbit and Rat *in Vivo*

From the above results, it was found that in addition to cyclohexylamine, cyclohexanone and cyclohexanol were detected as metabolites of CHS–Na in rabbit and rat. In order to study the formation processes of cyclohexanone and cyclohexanol, further investigation was carried out using cyclohexylamine.

On the gas chromatograms of sample solutions (G and H) (see Fig. 1), which were prepared from urine of rabbit and rat receiving cyclohexylamine as described in experimental, two peaks corresponding to cyclohexanone and cyclohexanol respectively were detected distinctly as shown in Fig. 5.

Accordingly, it was postulated that CHS–Na was metabolized primarily by the formation of cyclohexylamine, and the metabolite was oxidized further to afford the formation of cyclohexanone and cyclohexanol.

Further studies on these metabolites of CHS–Na are in progress.

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Synthesis of Epinephrine Monosulfates

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In the course of our studies on catecholamine metabolism, the need of the authentic catecholamine conjugates became evident. As to epinephrine, Richter's suggestion[2)] was given that the urinary epinephrine conjugate was a sulfate since its isolated conjugate from urine was positive for sulfate ester tests, but the lack of the authentic samples have obstructed the following progress. Now we wish to report the synthesis of two epinephrine monosulfates, namely epinephrine 3′–sulfate and epinephrine 4′–sulfate. The synthetic route is shown in Chart I.

1) Location: *Hongo–7–chome, Bunkyo–ku, Tokyo.*
2) D. Richter, *J. Physiol.* (London), **98**, 361 (1940).

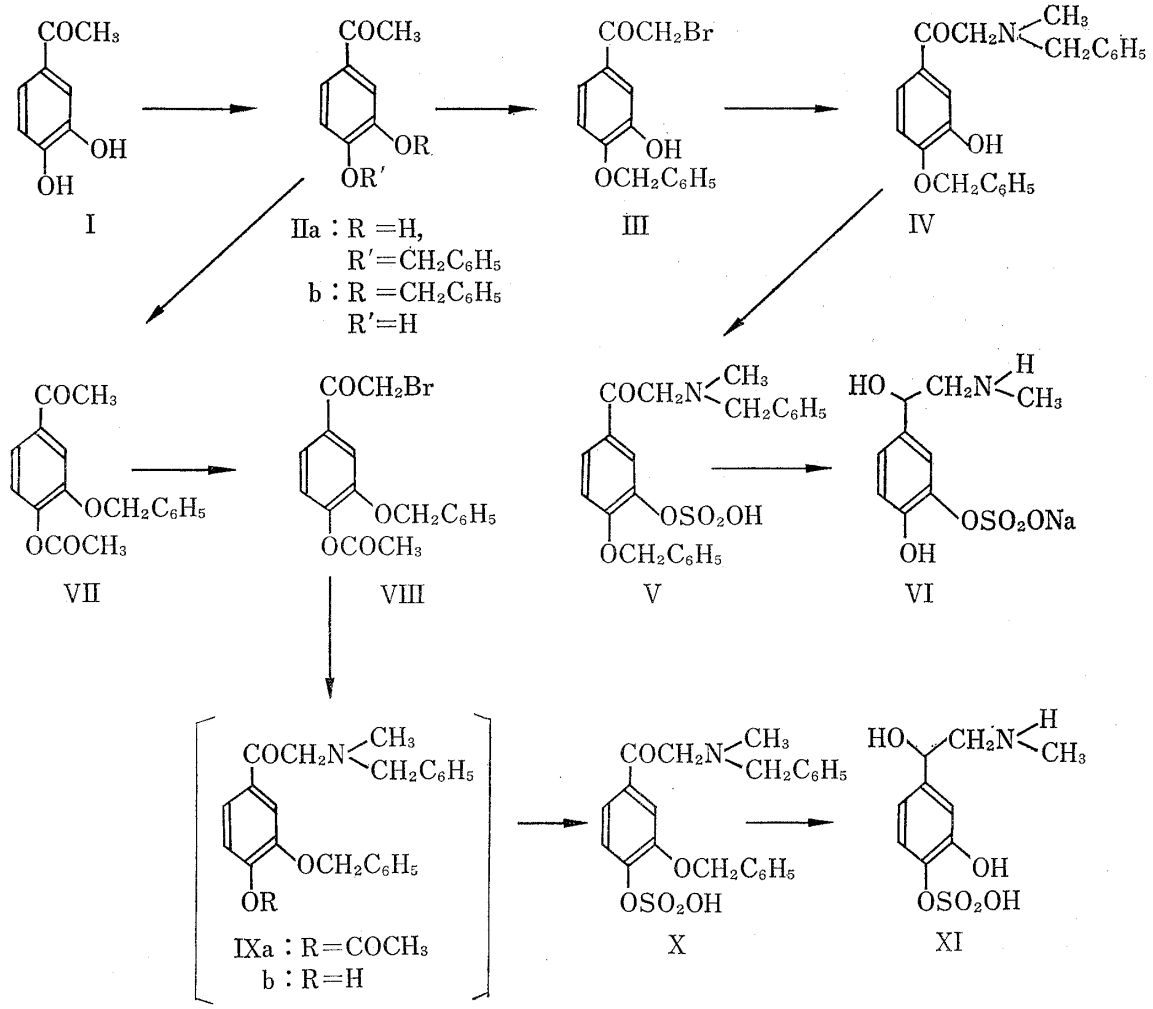

Chart 1

To protect one of the labile catecholic hydroxyl groups, 3',4'-dihydroxyacetophenone[3] (I) obtained from pyrocatechol diacetate was benzylated with equimolar benzyl chloride and sodium ethoxide in anhydrous ethanol, and the resulted monobenzyl ether derivatives, IIa and IIb, were differentiated from each other by several recrystallisation from ethanol. IIa coincided with the known specimen, 4'-benzyloxy-3'-hydroxyacetophenone.[4] The structure of IIb was determined by elementary analysis and the comparison of its NMR spectrum with that of IIa. As might be expected from inductive effect of acetyl group of I, the production ratio of IIa to IIb was about 4 to 1. IIa was brominated at the active methyl group with bromine in glacial acetic acid. The bromo derivative (III) was condensed with N-benzylmethylamine to give IV, which was crystallised as its hydrochloride. Sulfation of IV was achieved with chlorosulfonic acid and N,N-dimethylaniline in carbon disulfide.[5] The sulfated product (V) having characteristic S=O absorption band at 1040 cm^{-1} was led to catalytic hydrogenation over 5% palladium on charcoal to yield expected epinephrine 3'-sulfate (VI).

The step to gain epinephrine 4'-sulfate (XI) was attained with IIb as a starting material. Since the direct bromination of IIb gave 3'-benzyloxy-5'-bromo-4'-hydroxyacetophenone (mp 145—147.5°), protection of phenolic hydroxyl group was necessary. The acetylated derivative (VII) was brominated at the active methyl group, followed by condensation with

3) K.W. Rosemund and H Lohfert, *Chem. Ber.*, **61B**, 2601 (1928).
4) J. Hukki and E. Honkanen, *Acta Chem. Scand.*, **13**, 329 (1959).
5) E. Boyland and D. Manson, *J. Chem. Soc*, **1958**, 532.

N-benzylmethylamine to produce IXa, which was not crystallisable and deacetylated by heating with excess of N-benzylmethylamine. After isolation from the reaction mixture, IXb was submitted to sulfation as mentioned above. Hydrogenation of the sulfated product (X) afforded epinephrine 4′-sulfate (XI).

Experimental[6]

4′-Benzyloxy-3′-hydroxyacetophenone (IIa) and 3′-Benzyloxy-4′-hydroxyacetophenone (IIb)——IIa was obtained by the benzylation of 3′,4′-dihydroxyacetophenone[3] (I, 14.3 g) according to Hukki[4], *et al.*, as colorless needles, 5.5 g, mp 124—126° (reported[4] mp 119—120°). UV λ_{max}^{MeOH} mμ (log ε): 229 (4.19), 272 (4.05), 307 (3.86). NMR (in $CDCl_3$) τ: 2.47—2.65 (7H, aromatic proton), 3.08 (1H, aromatic proton), 4.84 (2H, $-OCH_2-$), 7.48 (3H, $-COCH_3$). From the mother liquor IIb was obtained as colorless needles, 1.4 g mp 140—142°. UV λ_{max}^{MeOH} mμ (log ε): 227 (4.27), 275 (4.01), 302 (3.89). NMR (in $CDCl_3$) τ: 2.40—2.63 (7H, aromatic proton), 3.05 (1H, aromatic proton), 3.82 (1H, –OH), 4.86 (2H, $-OCH_2-$), 7.47 (3H, $-COCH_3$). *Anal.* Calcd. for $C_{15}H_{14}O_3$: C, 74.36; H, 5.83. Found: C, 74.13; H, 5.82.

4′-Benzyloxy-2-bromo-3′-hydroxyacetophenone (III)——This was prepared according to Hukki[4], *et al.*, as colorless needles. mp 145—147°. (reported[4] mp 143—144°). UV λ_{max}^{MeOH} mμ (log ε): 233 (4.08), 280 (3.96), 315 (3.88).

2-N-Benzylmethylamino-4′-benzyloxy-3′-hydroxyacetophenone Hydrochloride (IV·HCl)——III was condensed with N-benzylmethylamine according to Hukki[4], *et al.* The reaction mixture was concentrated *in vacuo* and the oily residue was purified by preparative TLC on silica gel H with benzene–ether (1:2). The portion between *Rf* 0.4 to 0.7 was extracted with acetone and concentrated *in vacuo* to obtain oily residue (IV) which was dissolved in anhyd. ether and precipitated by the addition of HCl gas. The hydrochloride was crystallised from $CHCl_3$–ether as colorless prisms, mp 155—161.5° (decomp.). *Anal.* Calcd. for $C_{23}H_{23}O_3N\cdot HCl$: C, 69.42; H, 6.08; N, 3.53. Found: C, 69.60; H, 6.12; N, 3.49. UV λ_{max}^{MeOH} mμ (log ε): 233 (4.16), 281 (4.07), 316 (3.94).

2-Benzyloxy-5-(N-benzylmethylamino)methylcarbonylphenyl Sulfate (V)——The hydrochloride of IV (16 mg) was mixed with the solution of chlorosulfonic acid (40 mg) and N,N-dimethylaniline (0.4 ml) in carbon disulfide (2 ml). After standing at room temperature overnight, the reaction mixture was poured on ice water (2 ml) and the precipitate was crystallised from MeOH–H_2O as colorless prisms (4 mg), mp 198—200°. *Anal.* Calcd. for $C_{23}H_{23}O_6NS\cdot H_2O$: C, 60.12; H, 5.48; N, 3.05; S, 6.98. Found: C, 59.89; H, 5.33; N, 2.77; S, 6.94. UV $\lambda_{max}^{0.02NKOH\text{-}EtOH}$ mμ (log ε): 273 (4.12). IR ν_{max}^{KBr} cm^{-1}: 1042 (S=O).

Sodium Epinephrine 3′-Sulfate (VI)——V (64.5 mg) was hydrogenated over 5% palladium on charcoal (60 ml) in EtOH (40 ml) for 16 hr. The addition of 0.1% NaOH solution to the filtrate afforded precipitate, which was washed with EtOH. 20 mg mp 144° (bubbling)/260° (decomp.). *Anal.* Calcd. for $C_9H_{12}O_6NSNa\cdot 2H_2O$: C, 33.64; H, 5.02; N, 4.36. Found: C, 33.30; H, 4.90; N, 4.05. UV $\lambda_{max}^{0.02NKOH\text{-}EtOH}$ mμ (log ε): 246, (4.11), 297 (3.61), 336 (3.26). IR ν_{max}^{KBr} cm^{-1}: 1040 (S=O).

4-Acetyl-2-benzyloxyphenyl Acetate (VII)——IIb (39 mg) was acetylated with acetic anhydride (0.1 ml) and pyridine (0.2 ml) at room temperature overnight. The reaction mixture was treated as usual way and VII obtained was crystallised from aq. methanol as colorless prisms, (32.6 mg). mp 76—77°. *Anal.* Calcd. for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.83; H, 5.70. UV λ_{max}^{MeOH} mμ (log ε): 218 (4.41), 252 (3.98), 299 (3.54).

2-Benzyloxy-4-bromomethylcarbonylphenyl Acetate (VIII)——VII (1.03 g) was dissolved in glacial acetic acid (15 ml) and was brominated with a dropwise addition of bromine (578 mg) in glacial acetic acid solution (8.6 ml). After standing for 4 hr at room temperature the reaction mixture was poured on ice water and the precipitate was crystallised from MeOH as colorless needles (958 mg), mp 96—97°. *Anal.* Calcd. for $C_{17}H_{15}O_4Br$: C, 56.21; H, 4.19. Found: C, 56.22; H, 4.03. UV λ_{max}^{MeOH} mμ (log ε): 220 (4.66), 253 (3.91), 298 (3.51).

2-Benzyloxy-4-(N-benzylmethylamino)methylcarbonlyphenyl Sulfate (X)——The solution of VIII (100 mg) and N-benzylmethylamine (80 mg) in anhyd. benzene (3 ml) was left standing for 2 hr at room temperature. After addition of 100 mg of N-benzylmethylamine the reaction mixture was heated on a water bath for 3 min in order to cleave the acetyl group of IXa. The concentrated residue was submitted to TLC on silica gel H with benzene–ether (1:1). The acetone extract of adsorbent corresponding to *Rf* 0.42—0.61 was concentrated *in vacuo* to give oily residue (IXb, 34 mg), and the hydrochloride of IXb (16 mg) was mixed with the solution of chlorosulfonic acid (40 mg) and N,N-dimethylaniline (0.5 ml) in carbon disulfide (2 ml). After standing overnight at room temperature the ice water was added to the reaction mixture to give crystalline materials (4 mg) which were washed three times with MeOH, mp 127—134°. *Anal.* Calcd. for

6) All melting points were taken on a micro hot stage apparatus and uncorrected. IR spectra were measured with a Koken DS-402G Spectrometer, UV spectra with Carry 11, fluorescent spectra with Hitachi MPF-2 and NMR spectra with a JNM3H-60 Spectrometer using TMS as an internal standard.

$C_{23}H_{23}O_6NS \cdot 1\frac{1}{2}H_2O$: C, 58.96; H, 5.59; N, 2.99; S, 6.84. Found: C, 58.84; H, 5.29; N, 3.09; S, 6.23 (6.29). UV $\lambda_{max}^{0.02NKOH\text{-}EtOH}$ mμ (log ε): 259 (4.00), 302 (3.62), 348 (3.60). IR ν_{max}^{KBr} cm^{-1}: 1040 (S=O).

Epinephrine 4′-Sulfate (XI)——Hydrogenation of X (31 mg) was carried out with 5% Pd–C (44 mg) as the catalyst in EtOH for 16 hr. The reaction mixture was diluted with *n*–butanol (100 ml), filtrated, and concentrated *in vacuo* to give colorless semi crystalline materials (9.7 mg), which were washed with EtOH. mp 168—171°. *Anal.* Calcd. for $C_9H_3O_6NS \cdot \frac{1}{2}C_2H_5OH$: C, 41.95; H, 5.63; N, 4.89. Found: C, 42.05; H, 5.37; N, 5.11. UV $\lambda_{max}^{0.02NKOH\text{-}EtOH}$ mμ (log ε): 242 (3.98), 295 (3.66). IR ν_{max}^{KBr} cm^{-1}: 1038 (S=O).

Acid Hydrolysis of VI and XI——XI (1.58 μg) was dissolved in 0.1 ml of 0.1 N H_2SO_4, sealed in a glass tube *in vacuo* and heated on a water bath at 100° for 1 hr. After cooling, 0.05 ml of the solution was taken and 1 ml of acetate buffer (pH 6.0) and 0.1 ml of 0.25% solution of potassium fericyanide were added. After 2 min, 0.9 ml of 20% NaOH and 0.1 ml of 0.2% ascorbic acid solution were added to the reaction mixture, which was submitted to fluorometry at an excitation wave length of 410 mμ and an emission wavelength of 510 mμ. The observed liberated epinephrine value (0.37 μg) coincided with the assumed one (0.37 μg) from degradation under the same condition, and its excitation and emission spectra were identical with that of the authentic epinephrine. The simillar result was obtained with VI.

The hydrolysate of XI with 0.1 N HCl was led to trifluoroacetyl derivative[7] and submitted to GLC using Shimadzu GC–4APE. The chromatogram showed the same retention time as the authentic epinephrine derivative.

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7) S. Kawai and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **16**, 1091 (1968).