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Metabolism of Methylscopolammonium Methylsulfate (DD-234) in Rats[1]

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The metabolism of methylscopolammonium methylsulfate (I) in rats was investigated. Eight metabolites were detected in urine and faeces after oral and subcutaneous administrations. The feature of metabolic pathway was characterized as follows; i) the quarternary ammonium moiety was unchanged, ii) major pathway involved oxidation at the *para*-position of benzene ring to introduce a hydroxy and a methoxy groups, iii) minor products were the apo-derivative, glucuronide of I and scopine derivative. Absorption and excretion were examined by using ^{14}C-labeled compound, and excreted total radioactivities were 82.2% of oral dose and 93.6% of subcutaneous dose in urine and faeces. In respiratory air $^{14}CO_2$ was not detectable. Comparison of the metabolic pathway with that of tertiary scopolamine are also discussed.

Methylscopolammonium methylsulfate (DD-234) (I) is a quarternary ammonium salt of scopolamine (II) and has been developed as an anticholinergic agent with a weaker ganglionic blocking effect than that of butylscopolammonium bromide.[3] Toyoshima, *et al.*[4] have reported the tissue distribution of DD-234-$^{14}CH_3$ in mice and Suga[5] has also investigated the absorption, distribution and excretion in rats and mice. An important information from these studies is that the radioactivities due to DD-234-$^{14}CH_3$ were distributed mainly in the excretory and the circulatory organs but only poorly in the brain and other organs. In addition, the absorption and excretion of I were observed to be faster than those of scopolamine and the results are ascribed to the properties of a quarternary ammonium ion of I. Autoradiograph of the quarternary ammonium derivative of scopolamine and scopolamine itself in mice have

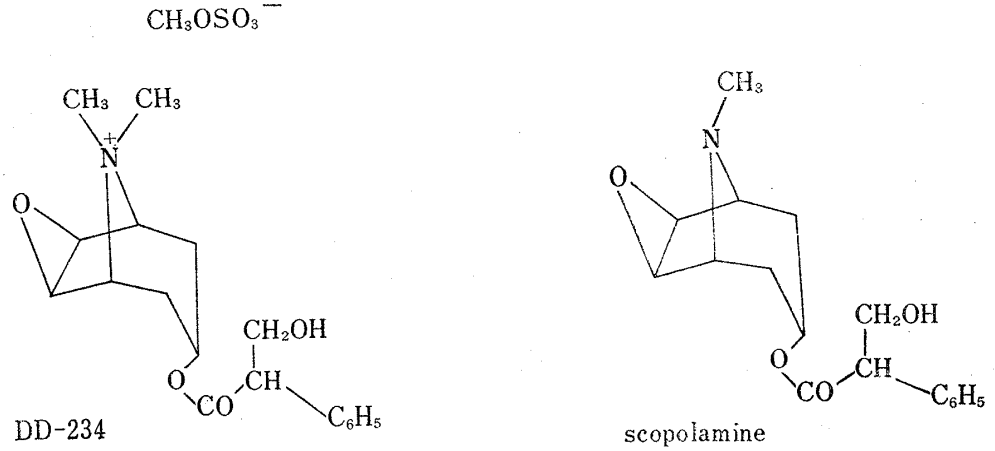

Chart 1

1) This work was presented at the 91st Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April, 1971.
2) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
3) A. Kasahara, H. Kojima, T. Onodera, T. Hashizume, T. Yamasaki, K. Tawara, and T. Chiba, *Folia Pharmacologica Japonica*, **67**, 100 (1971).
4) S. Toyoshima, T. Seto, H. Fujita, N. Tonegi, M. Naganuma, and Y. Nakamura, *Yakubutsu-Ryoho*, **3**, 2143 (1970).
5) T. Suga, *Clinical Report*, **4**, 2617 (1970).

been investigated by Shindo, *et al.*[6] and similar results due to the quarternary ammonium salt have also been obtained. These studies suggested that there were marked differences between the metabolic fate of a tertiary amine and that of its quarternary ammonium salt. The structure determination of metabolites of scopolamine and other tertiary amines of tropane series have already been carried out by Werner, *et al.*[7] but metabolites of their quarternary ammonium salts have not been studied. In the present paper, we wish to report the elucidation of structures of metabolites of I and the urinary and faecal excretion of the metabolites following oral or subcutaneous administration to rats.

Material and Method

General Analytical Procedures——Measurement of Total Radioactivity: Urine samples diluted with water to an appropriate concentration were pipeted into 15 ml of a scintillation fluid, which was prepared by dissolving 0.30 g of dimethyl POPOP, 7.0g of PPO and 100 g of naphthalene in 900 ml of dioxane. All activities were corrected for quenching. Radioactivity was determined with an Aloka LSC-601 liquid scintillation counter. The radioactivity of faeces was determined by a combustion method as follows; about 50 mg sample of dry faeces was combusted by using a TRI-CARB Sample Oxidizer, and the resulting $^{14}CO_2$ was absorbed in ethanolamine. The ethanolamine solution, MeOH and a scintillation fluid, which was prepared by dissolving 15 g of PPO, and 1 g of bis-MSB in 1 liter of toluene, were mixed in a ratio of 3: 9: 7 in volume. Radioactivity of the mixture was determined as given above. The counts were corrected by preliminary test for the quenching effect (^{14}C counting efficiency: 90%).

Thin-Layer Chromatography (TLC) and Paper Partition Chromatography (PPC): Glass plates (5×20 cm) for TLC coated with a layer (0.25 mm) of Silica gel GF_{254} (Merck) and activated at 105° for 1 hr were stored in desiccator over silica gel. Toyo Roshi No. 51 was used for PPC. The chromatograms were developed by ascending or descending method with the following solvent system; (A) MeOH, (B) *n*-BuOH–AcOH–H_2O (3: 1: 2), (C) *n*-BuOH–AcOH–H_2O (5: 1: 5) (uppr elayer). Dragendorff's reagent was used to visualize the spots.

Measurement of Radio-scannogram of TLC and PPC: Radio-scannogram of TLC and PPC were taken on Aloka TL chromatogram scanner Model TLC-2B, and Aloka PP chromatogram scanner Model TRM-1B, respectively.

Other Spectral Analysis: Analytical spectrum measurements were carried out with following instruments. JEOLCO JNM 4H-100 nuclear magnetic resonance (NMR) spectrometer (100 MHz), Hitachi R-20B NMR spectrometer (60 MHz), Hitachi EPI-G_2 infrared (IR) spectrometer and Hitachi 124 spectrometer.

Material——^{14}C-Labeled methylscopolammonium methylsulfate (DD-234-$^{14}CH_3$) was synthesized from scopolamine by quarternarization in ethanol with ^{14}C-labelled dimethylsulfate. The product, of which the specific radioactivity was 9.5 mCi/mmole, shows two radioactive spots on the TLC developed by solvent system A. The spots (*Rf* 0.85, 0.10), which have almost similar peak area on the TLC, are attributable to methylscopolammonium ion and methylsulfate ion, respectively. Other radioactive impurities were not detectable in the product. The product was diluted with cold DD-234 to the appropriate specific radioactivity according to the purposes of the individual experiments.

Animals——Male rats of Wistar strain weighing approximately 200 g were used.

Administration of the Drug——Oral Administration: The oral doses were administered 1 ml per rat of an aqueous solution of DD-234-$^{14}CH_3$ containing 50 mg/16 μCi/ml or 3.14 mg/1 μCi/ml. For the preparative separation of the metabolites, a dose of 1500 mg/kg of I without radioactivity (150 mg/ml aqueous solution) was given orally each of 20 rats.

Subcutaneous Administration: Two kinds of preparations (A and B) were used for the subcutaneous injection. Preparation A consists of 7.5 mg (167 μCi) of DD-234-$^{14}CH_3$, 8.8 mg of citric acid, 90 mg of NaCl and 10 ml of water, and was finally adjusted to pH 4.30±0.01 with 0.1 N NaOH, exactly. Preparation B, for preparative separation of metabolites, was also obtained from 2 g of I, 17 mg of citric acid, 50 mg of NaCl and 20 ml of water in a similar way. A dose of 3.75 mg/kg of preparation A or B was administered hypodermically.

Collection of Urine and Faeces——Urine was collected during three periods, 0—24, 24—48, and 48—72 hr, and faeces were also collected for 0—72 hr.

Collection of the $^{14}CO_2$ in Respiratory Air——After oral administration of DD-234-$^{14}CH_3$ a rat was kept for 48 hr in a desiccator equipped with outlet and inlet tubes, and $^{14}CO_2$ in the air stream passed through

6) H. Shindo, I. Takahashi, and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **19**, 513 (1971).
7) *a*) G. Werner and K.H. Schmidt, *Hoppe-Seyler,s Z. Physiol. Chem.*, **349**, 741 (1968); *b*) G. Werner and K.H. Schmidt, *Hoppe-Seyler's Z. Physiol. Chem.*, **349**, 677 (1968).

the desiccator was absorbed in 10% aqueous ethanolamine solution. Radioactivity of the ethanolamine solution was determined in a scintillation fluid of toluene system, which was described in general analytical procedure.

Result

I. Detection for the Urinary Metabolites

Thin-Layer Chromatography——Concentrated urine collected during 0—24 hr after oral administration was directly subjected to TLC with solvent B in ascending system. I and its metabolites were detected by radioscanner and by Dragendorff's reagent, which developed orange yellow color with tropan skeleton. As shown in Fig. 1, several yellow spots by Dragendorff's reagent and three radioactive spots were revealed on the plate. As mentioned above, the quarternary ammonium salt, which was labeled on both of the anion and the cation, showed two radioactive spots on the TLC. This suggests that the both ions separately move on the silica gel plates. On the basis of its behavior, the radioactive spot having the largest *Rf* value, and not colouring with Dragendorff's reagent, proved to be the spot of the methylsulfate ion component. Consequently, the lower two spots (F-I, F-III) was attributed to the metabolites arising from the part of methylscopolammonium ion (abbreviated as MH-skeleton) of I.

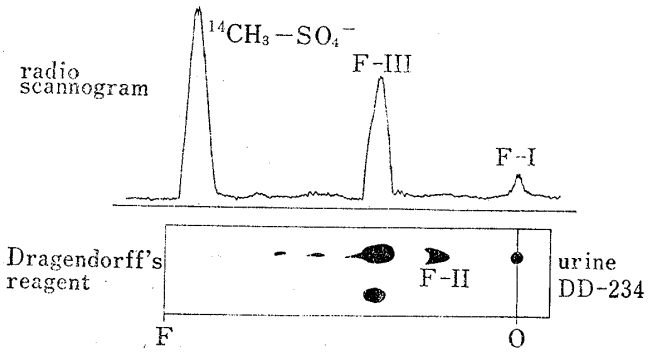

Fig. 1. TLC Pattern of Metabolites in Urine Collected during 0—24 hr

developed by solvent system B, ascending

Further TLC conditions were investigated in detail by using several solvent system in order to obtain a good separation of the metabolites. F-III fraction gave further four spots (B, C, D and E) in the solvent C in descending system after 20 hr-developing, and F-I also gave two spots (M-I and A) in the same way, as shown in Fig. 2. These metabolites were detectable by Dragendorff's reagent and radioactivity.

Paper Partition Chromatography——F-III fraction was also examined by the PPC using the solvent B in ascending system. The similar result with that of TLC was obtained, and no more metabolic spots other than 6 compounds were detected on the paper chromatogram.

The pattern of TLC and PPC of urine after subcutaneous administration were quite similar with the above results of oral administration.

II. Separation of the Urinary Metabolites and Unchanged DD-234

DD-234 has been found to be hydrolyzed extremely rapidly in alkaline media giving methylscopinium methylsulfate (III). Similar observation is known in scopolamine methyl bromide.[8] Therefore, special precaution for protecting the ester linkage from alkali was taken in the treatment of the biological sample. Another difficulty in the separation of the biotransformed products was that the presumable metabolites, if they were quarternary ammonium salt, would be insoluble in organic solvents other than alcohols.

The separation of the metabolites and unchanged I from the urine collected during 0—48 hr after oral and subcutaneous administration to 20 rats was carried out by the following method. Urine was concentrated to dryness under 30° *in vacuo*, and the resulting residue was extracted with methanol (170 ml). The insoluble material (F-I), of which TLC pattern was similar to that shown in Fig. 2, was obtained as powdered substances (4.5 g). The methanolic extract was concentrated *in vacuo* to obtain a syrupy material (13.4 g), which was

8) R.B. Moffett and E.R. Garrett, *J. Am. Chem. Soc.*, 77, 1245 (1955).

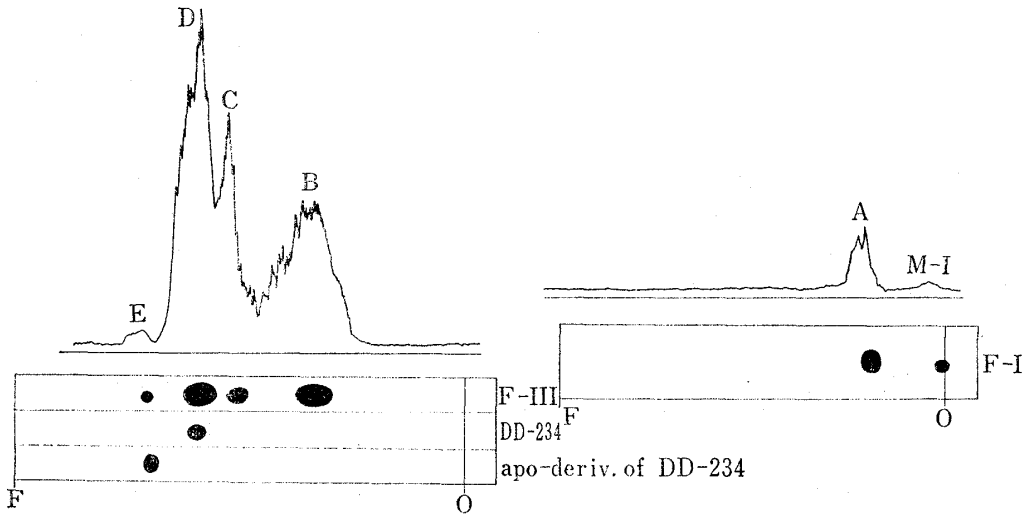

Fig. 2. Radio Scannogram on TLC of F–I and F–III from Rat Urine after Oral Administration

developed by solvent system C, descending
detected by Dragendorff's Reagent

separated through Amberlite IRF-97 cation resin. A column (*ca.* 5 cm in diameter) containing about 500 ml of the resin in hydrogen form was used. The crude syrupy material was dissolved in a small amount of water, passed through the column and washed thoroughly with water and eluted with 0.05N HCl. The water washings and 0.05N HCl eluate were named as F-II and F-III, respectively. In the preliminary experiment using radioactive compound, radioactivity was hardly recognized in the fraction corresponded to F-II, but observed in the eluate corresponded to F-III, mainly. F-III gave the same radio scannogram as Fig. 2. As seen from the figure metabolite B, C, D and E are effectively collected in F-III. The procedure of the separation of the metabolites from rat urine is summarized in Chart 2.

For further separation of 6 metabolites from F-I and F-III, several methods were examined. However, because of the specific character of the quarternary ammonium salt,

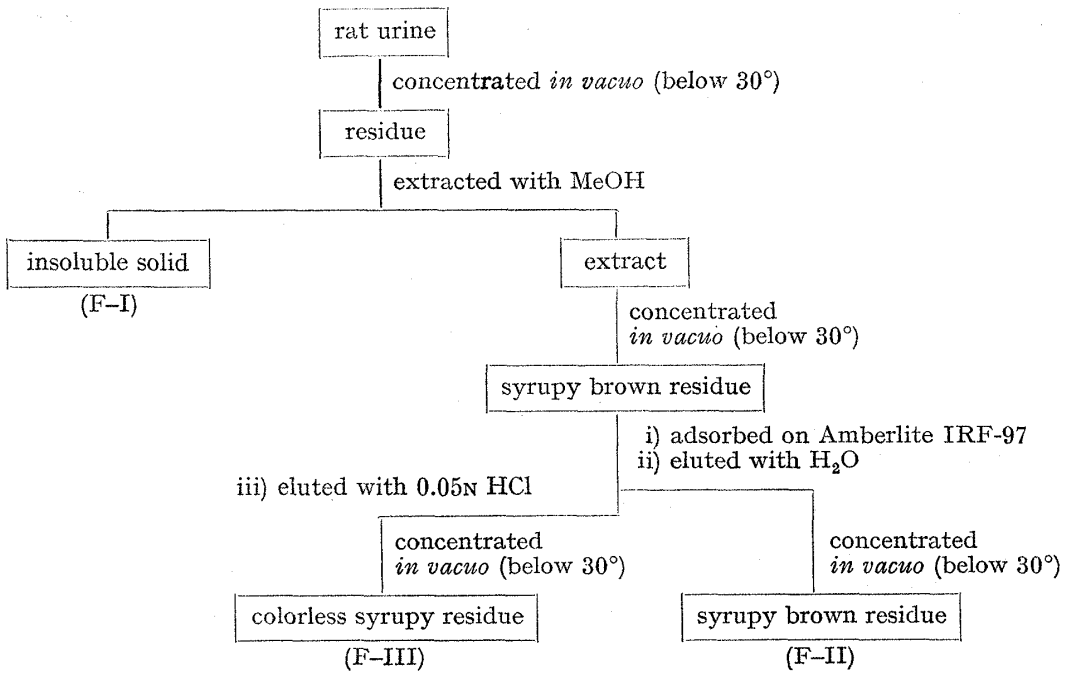

Chart 2. Separation of Metabolites from Rat Urine

there were no other satisfactory procedures for the isolation of metabolites than a preparative TLC or partition chromatography. Metabolite B and C were purified by the partition chromatography using Celite 545 impregnated with 0.05N HCl as stationary phase and *n*-BuOH as mobile phase.

Metabolite M-I and A were obtained from F-I fraction by the preparative TLC developed by the solvent B in descending system. Metabolite B, C, D and E were also isolated from F-III by the essentially same way as in the case of F-I, followed by further purification using a partition chromatography, of which the column consisted of Celite 545 containing 0.05N HCl as stationary phase and *n*-BuOH as mobile phase.

III. Structure of the Urinary Metabolites

Metabolite M-I——Metabolite M-I, whose *Rf* value was close to zero in any solvent system, was assumed as a glucuronide. Therefore, the enzymatic hydrolysis of the metabolite M-I with β-glucuronidase was followed. M-I (25 mg) solution in 0.05M-phosphate buffer (pH 7.0) was mixed with β-glucuronidase (20 mg, 1000 units) and the mixture was incubated at 37° for 24 hr. The reaction mixture was concentrated to dryness *in vacuo* and extracted with methanol. The solvent of the extract was evaporated *in vacuo*, and the residue was dissolved in water again. The aqueous solution was treated with Amberlite IRF-97 (10 ml) cation resin (hydrogen form). The crystalline material obtained from the 0.05N HCl eluate of the resin column was recrystallized from the mixed solvent of ethanol and diisopropyl ether to give colourless prisms mp 199—201°. IR and NMR spectra of the product were identical with those of methylscopolammonium chloride. Also, the melting point of a mixture with an authentic sample was not depressed. The above results was interpreted as indicating metabolite M-I to be the glucuronide of DD-234.

Metabolite A——Metabolite A, which was one of minor metabolites, seemed to be a glucuronide, because of its small mobility on the TLC in any solvent system. However, scarcity of material prevented further examination, and the structure was not completely determined.

Metabolite B——Metabolite B was recrystallized from a mixture of ethanol and diisopropyl ether to give prisms, mp 213—215° (decomp.), as chloride. The NMR spectrum (Fig. 3) of the metabolite was analogous to that of I except the presence of the characteristic signals of a *p*-substituted phenyl group, which appeared in 6.9 and 7.3 ppm as A_2B_2 qualtet (J=8 Hz), as shown in Fig. 3. The profile of the aromatic component to be *p*-hydroxyphenyl derivative was supported by comparison of ultraviolet (UV) spectra of the metabolite and *p*-hydroxy-

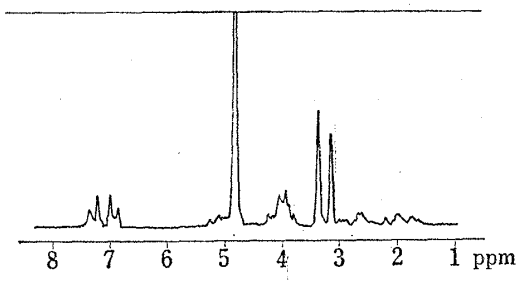

Fig. 3. NMR Spectrum of Metabolite B (in D_2O, 60 MHz)

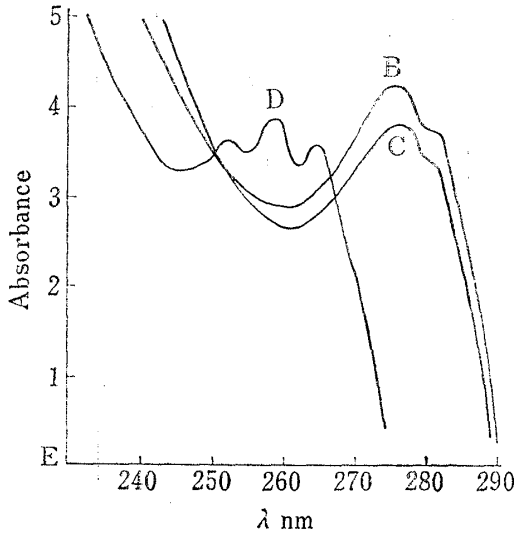

Fig. 4. Ultraviolet Spectra of Metabolite B, C and D (in H_2O)

toluene as shown in Fig. 4. In the IR spectrum the characteristic absorptions were observed at 1715 cm^{-1} and at 912 cm^{-1} and 860 cm^{-1} indicating the presence of an ester linkage and an epoxide group, respectively. Hydroxylation of the metabolite with alkali, followed by similar treatment as described by Moffett, *et al.*[8] gave methylscopinium chloride, which was identified with an authentic sample prepared by the standard method.[9] Consideration of the above results led to the assignment of the 4′-hydroxymethylscopolammonium chloride for metabolite B. (*Anal.* Calcd. for $C_{18}H_{24}O_5NCl$: C, 58.41; H, 6.54; N, 3.79. Found: C, 57.96; H, 6.37; N, 4.02).

Metabolite C——Metabolite C was obtained as glassy substance which could not be crystallized despite any efforts. The characteristic signals in the NMR spectrum appeared at 6.9 and 7.1 ppm (quartet, 4H, J=8 Hz) and 3.8 ppm (singlet, 3H). The former was assigned to *p*-substituted phenyl protons, similarly to the observation in the case of metabolite B, and the latter corresponded to the signal of a methoxy group. Other part of the NMR pattern was almost similar to that of I. The UV spectrum of the metabolite gave another evidence for the presence of *p*-methoxyphenyl group as shown in Fig. 4. The IR spectrum showed the bands at 1715 cm^{-1} corresponding to a carbonyl of ester and at 912 and 860 cm^{-1} to an epoxide. Treatment of the metabolite with alkali converted to the methylscopinium salt, which was identified with an authentic sample described in the metabolite B. From the above results, metabolite C was presumed to be 4′-methoxy-methylscopolammonium chloride, and this was confirmed by high-resolution mass spectrometry.

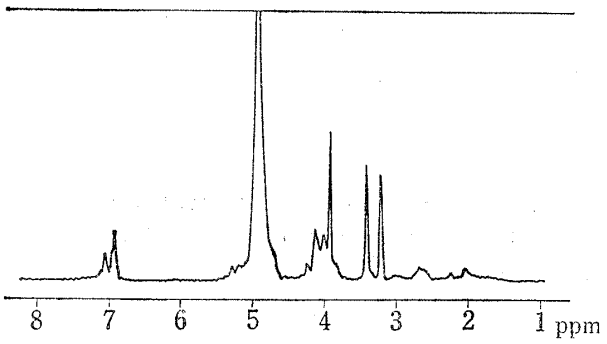

Fig. 5. NMR Spectrum of Metabolite C (in D_2O, 60 MHz)

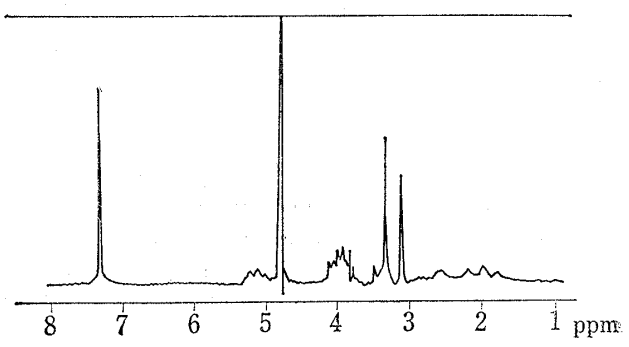

Fig. 6. NMR Spectrum of Metabolite D (in D_2O, 60 MHz)

Metabolite D——Metabolite D was recrystallized from ethanol and diisopropyl ether to get prisms, mp 199—201°. The IR, NMR and UV spectra of the compound were identical with those of methylscopolammonium chloride. This proved that supposed metabolite D was unchanged I.

Metabolite E——Metabolite E was not isolated in a completely pure form because of its minute ammount. By comparison of its pattern of TLC and PPC with those of several standard samples which were expected as the metabolites, the *Rf* values of metabolite E in several solvent system were shown to be coincident with those of methylaposcopolammonium chloride prepared by the established procedure.[10] The structure was finally clarified by an isotope dilution method.

IV. Detection and Identification for the Faecal Metabolites

Dry faeces from orally or subcutaneously dosed rats were powdered and extracted with water. Most of radioactivity in faeces was effectively extracted with water. The extracts were examined by TLC on the basis of the knowledge of detection obtained in the case of the

9) E.R. Garrett, *J. Am. Chem. Soc.*, **79**, 1071 (1957).
10) R.B. Moffett and B.D. Aspergren, *J. Am. Chem. Soc.*, **78**, 3448 (1956).

urinary metabolites. TLC pattern of the faecal metabolites from subcutaneous administration was quite similar to that of urine except presence of two new radioactive spots named metabolite F and G, as shown in Fig. 7b. But in the case of faeces from oral dose, only two radioactive spots were detectable (Fig. 7a), one of which coloured orange yellow and the other violet by Dragendorff's reagent. These spots were attributable to metabolite D and F, respectively.

The separation of the metabolites from the faeces collected during 0—48 hr was achieved by the method using an ion exchange chromatography as described in Chart 3.

Metabolite F——Metabolite F was unable to be crystallized due to the scarcity of material in spite of being chromatographically pure. But the metabolite was presumed as methyl-

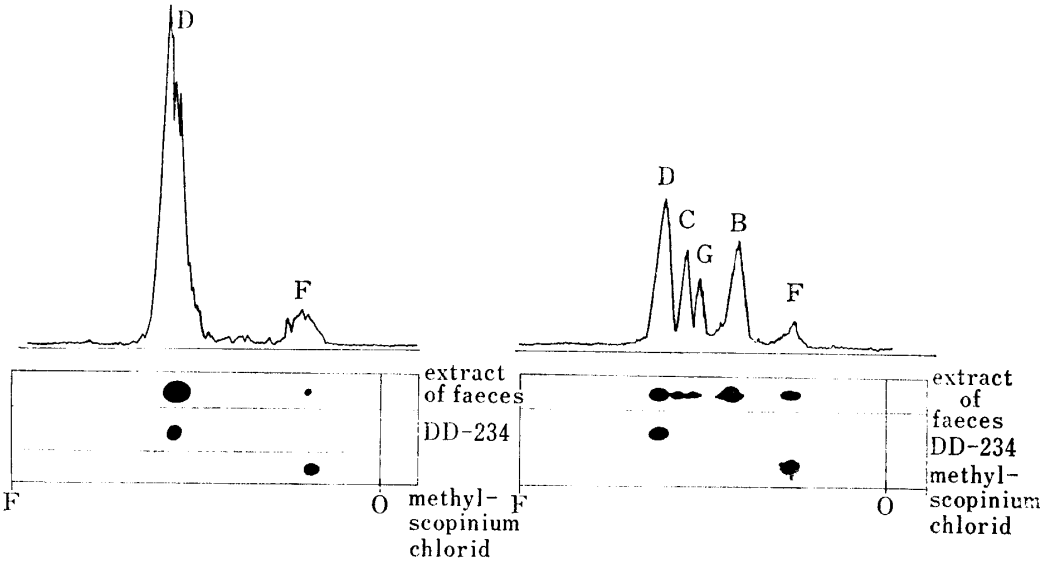

Fig. 7a. Radio Scannogram on TLC of Extract of Faeces from Oral Administration

Fig. 7b. Radio Scannogram on TLC of Extract of Faeces from Subcutaneous Administration

developed by solvent system C, descending 20 hr
detected by Dragendorff's reagent

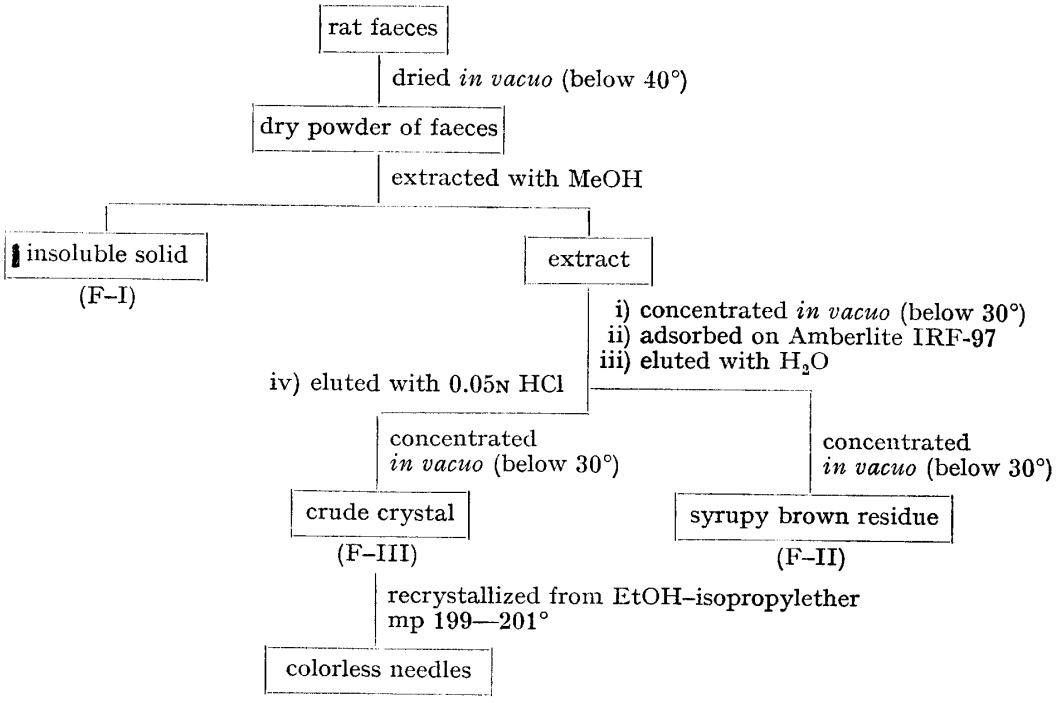

Chart 3. Separation of Metabolites from Rat Faeces

scopinium chloride by the *Rf* value of TLC, and was identified by an isotope dilution method using recrystallizations four times from ethanol–aceton.

Metabolite G——Metabolite was expected to be an analogous compound with 4′-hydroxy derivative, but scarcity of material prevented further examination, and the structure was not completely determined.

VI. Absorption and Excretion of I

The estimation of the absorption through the alimentary canal and excretion of I was accomplished on the basis of the results in the studies on the metabolites.

Table I illustrates the excreted total radioactivity in urine and faeces after oral (81 μCi/rat) and subcutaneous (20.4 μCi/rat) administration, and total excreted activities were 66.8 μCi (82.2% of dose) and 19.1 μCi (93.6% of dose), respectively, during 0—48 hr.

Table I. Excreted Radioactivity in Urine and Faeces after Oral and Subcutaneous Administration of DD-234-$^{14}CH_3$ μCi (%)

Administration and doses	Ions[a]		In urine			In faeces	Total
			0—24 hr	24—48 hr	Total	0—48 hr	
Oral	$^{14}CH_3$–NH^+	40.5 (100)	5.8 (14.4)	1.9 (4.6)	7.7 (19.0)	28.0 (69.2)	35.7 (88.2)
	$^{14}CH_3$–SO_4^-	40.5 (100)	28.6 (70.6)	1.7 (4.2)	30.3 (74.8)	0.6 (1.4)	30.9 (76.3)
	Total	81.0 (100)	34.4 (42.5)	3.6 (4.4)	38.0 (46.9)	28.6 (35.3)	66.6 (82.2)
Subcutaneous	$^{14}CH_3$–MH^+	10.2 (100)	5.7 (55.9)	0.15 (1.5)	5.9 (57.8)	2.5 (24.5)	8.4 (81.9)
	$^{14}CH_3$–SO_4^-	10.2 (100)	10.6 (103.9)	0.14 (1.3)	10.7 (104.9)	0.0 (0.0)	10.7 (104.9)
	Total	20.4 (100)	16.3 (79.9)	0.29 (1.4)	16.6 (81.3)	2.5 (12.3)	19.1 (93.6)

a) DD-234-$^{14}CH_3$=$^{14}CH_3$-MH^++$^{14}CH_3$-SO_4^-

In order to clarify whether the excreted radioactivity was originated from MH-skeleton or from methylsulfate ion, the following TLC examination were carried out. As shown in Fig. 1, the solvent system B was convenient for separation of the methylsulfate ion from the others. The ratio of the both part in urine and faeces was calculated from peak areas of scannogram of radioactivity of TLC. As summarized in Table I, MH-skeleton part eliminated in urine during 0—48 hr was 19.0% of an oral dose, and the 69.2% was excreted in faeces. On the contrary, most part of methylsulfate ion (74.8% of dose) appeared in urine and only 1.4% was in faeces. In the case of subcutaneous administration, 57.8% of MH-skeleton part was excreted in urine and 24.5% of it was recognized in faeces, but methylsulfate was detected only in urine. These results suggested that MH-skeleton was considarably excreted in bile, and similar observation in the case of scopolamine-N-butyl bromide was reported.[11] Therefore, actual absorption ratio of MH-skeleton in oral administration can be estimated as about 30% of an oral dose (by assuming that one third of absorbed drug is excreted into bile).

The ratio of the metabolites and unchanged drug excreted in urine and faeces during 0—48 hr after oral and subcutaneous administrations are summarized in Table II. The results were obtained by the calculation of the peak area of scannogram of TLC, developed by the solvent system C as shown in Fig. 2 and 7.

In the urine, the pattern of the metabolic transformation after oral administration was essentially identical to that after subcutaneous injection, except in a few trifling matter.

11) T. Oguma, T. Muramatsu, T. Iga, T. Fuwa, S. Awazu, and M. Hanano, *Chem. Pharm. Bull.* (Tokyo), **21**, 1554 (1973).

Table II. Ratio of Metabolites and Unchanged Drug in Urine and Faeces after Administration of DD-234-$^{14}CH_3$

Administration	Metabolite												
	In faeces					In urine							
	B	C	D	F	G	M-I	A	B	C	D	E	F	G
Oral	0	0	91.7	8.3	0	1	5.4	39.6	11.6	34.9	8.4	0	0
Subcutaneous	32.8	17.2	30.0	8.0	8.6	11.0	2.4	13.6	13.8	49.9	1.6	5.5	2.2

Table III. Radioactivity of $^{14}CO_2$ in Respiratory Air of Rat after Oral Administration of DO-234-$^{14}CH_3$

Sampling time hr	0—1	1—2	2—3	3—4	4—5	5—6	6—7	7—8	8—9	9—24	Total
Radioactivity $\times 10^{-3}$ μCi	2.8	2.1	1.7	1.5	1.2	1.5	1.1	1.1	1.3	0.7	25.0

Major compounds in urine were the unchanged drug and the 4′-hydroxy and 4′-methoxy derivatives.

DD-234-$^{14}CH_3$ was administrated orally to a rat, and $^{14}CO_2$ in respiratory air was also measured. The results are summarized in Table III. Total excreted radioactivity within 24 hr after administration was only 0.025 μCi (0.03% of an oral dose). The results suggested that labeled methyl group on MH-skeleton was hardly eliminated as CO_2 and also were consistent with the finding that *nor*-derivatives of I was not detected in urinary metabolites.

Discussion

Metabolic pathway of I in oral and subcutaneous administration is shown in Chart 4. With respect to the structural fate of I, the followings were thought to be interesting; i) the quarternary ammonium moiety was unchanged, ii) in the major pathway oxidation at the *para*-position of benzene ring took place to introduce a hydroxy and a methoxy groups, giving the metabolite B and C, respectively, iii) minor products were the apo-derivative (metabolite E), the glucuronide (metabolite M-I) of the unchanged drug and the scopine derivative (metabolite F) which was provided by the cleavage of the ester linkage. These results showed a very distinct contrast in the feature of metabolic pattern to that of tertiary scopolamine which was investigated by Werner.[7a] The major metabolites of the tertiary amine are the 6-hydroxy compound resulted from the reductive cleavage of the epoxide ring and the *nor*-compound produced by the oxidative demethylation of N-CH_3 group, whereas those of quarternary amine are the 4′-hydroxy and 4′-methoxy compounds. These differences may be attributed to fundamental chemical properties of quarternary and tertiary amines.

The chemistry of scopolamine shows that its quarternary ammonium derivative is rapidly hydrolyzed under mild alkaline condition to yield quarternary scopine and tropic acid while scopolamine is slowly hydrolyzed to scopoline and tropic acid. This suggests that the quarternarization of scopolamine stimulates fission of the ester linkage whereas inhibits cleavage of the epoxide ring. In addition, the primary alcoholic hydroxyl group in the case of quarternary amine is readily acetylated and concomitantly eliminated by deacetoxylation to give an apo derivative.

If these chemical reactivities of the tertiary and the quarternary amine were directly correlated with their metabolic fate, scopine derivatives and apo derivatives would be formed from the quarternary amine more than those from the tertiary amine. In the present experiments of I, such derivatives were not determined in appreciable amounts compared to

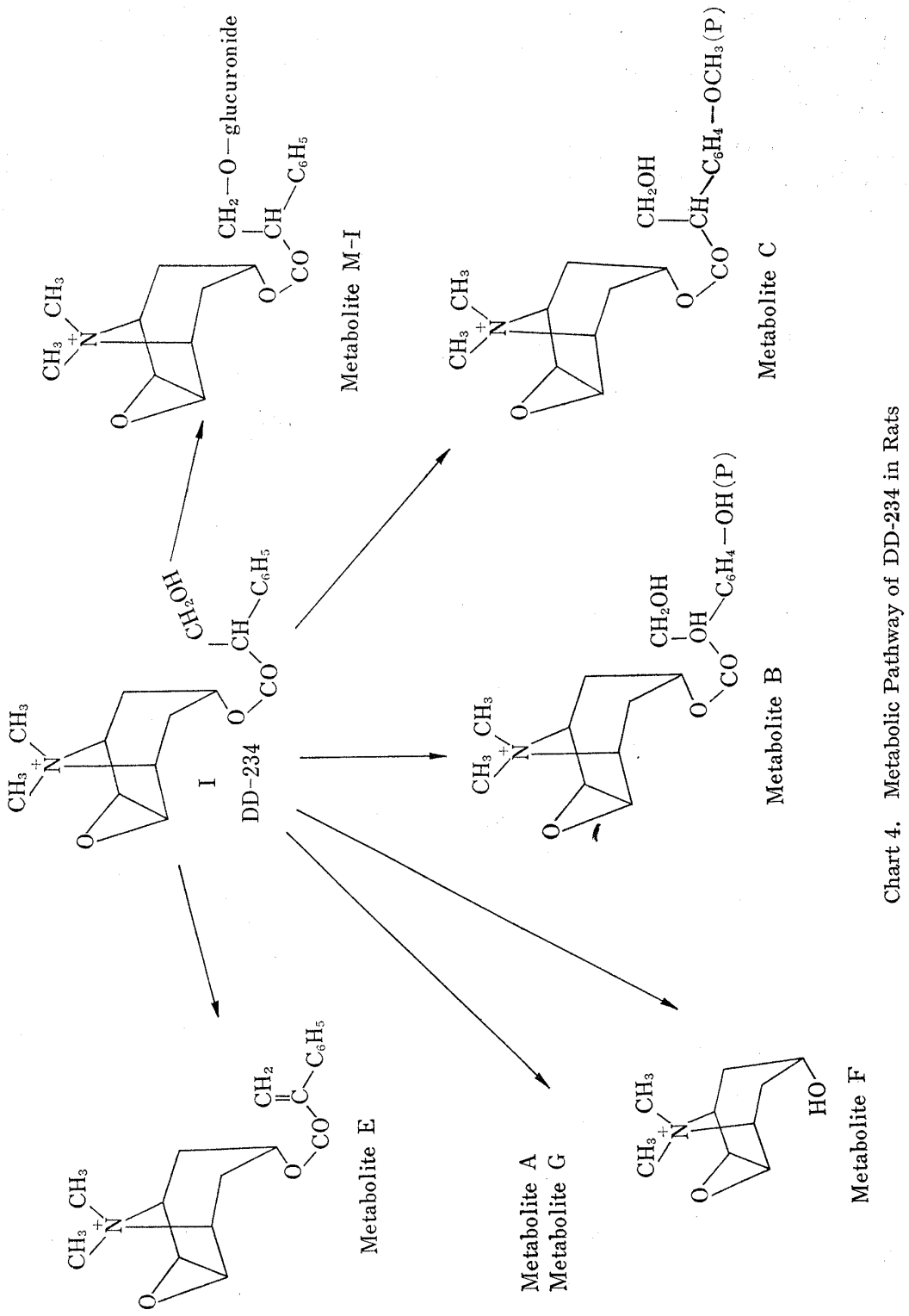

Chart 4. Metabolic Pathway of DD-234 in Rats

the case of scopolamine. The results showed that the susceptibility of the ester linkage to drug metabolizing enzymes was different between the tertiary and the quarternary amine.

Atropin, a tertiary amine without an epoxide ring, is metabolized to give the 4′-hydroxy compound and scopolamine is transformed to the 6-hydroxy metabolite.[7b] In connection with these facts, introduction of the 4′-hydroxy and 4′-methoxy groups in the metabolism of I is probably associated with inhibited cleavage of the epoxide ring.

As one of mechanisms of demethylation of N-methyl groups to produce a *nor*-compound, oxidative decarboxylation passing through the N-oxide is considered. In the case of I, forma-

tion of N-oxide will be unable because of its quarternary structure of the amino group. However, the lack of the *nor*-derivative of I in the metabolites is preferably explained in terms of Brodie's view[12] that the N-methyl group of polar compounds is generally not subjected to oxidative demethylation.

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12) L.E. Gandette and B.B. Brodie, *Biochem. Pharmacol.*, 2, 89 (1959).