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Synthesis and Metabolism of 2,2-Dimethylnonadecanoic Acid *

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The intestinal absorption and metabolism in the rat of 2,2-dimethylnonadecanoic acid has been studied. The main metabolic end-products were 2,2-dimethylglutaric acid and 2,2-dimethylpimelic acid, which were recovered in the urine. Thus the β-oxidation from the carboxyl end of the 2,2-dimethylnonadecanoic acid had been completely hindered and the last step of the degradation from the ω end — from 2,2-dimethylpimelic to 2,2-dimethylglutaric acid — had been retarded by the steric hindrance of the two methyl groups.

The intestinal absorption and metabolism of 2,2-dimethylstearic acid in the rat has been studied in an earlier investigation by Bergström, Borgström, Tryding and Westöö [1]. The acid was well absorbed. The β-oxidation from the carboxyl end of the 2,2-dimethylstearic acid molecule was completely hindered and the main metabolic end product was shown to be 2,2-dimethyladipic acid which was excreted in the urine.

Continuing the study on the influence of steric hindrance at the carboxyl group, we have now investigated the absorption and metabolism of a 2,2-dimethyl fatty acid with an odd number of carbon atoms in the chain. For this reason we have prepared 2,2-dimethyl[1-^{14}C]nonadecanoic acid by a Kolbe electrolysis of heptadecanoic acid and 3-methoxy[^{14}C]carbonyl-3-methylbutyric acid, purification of the methyl ester obtained by chromatography on acid alumina and hydrolysis of the ester.

When administered in olive oil solution *per os* to rats, the acid was well absorbed. The absorbed activity ** was recovered in the urine to 78% (mean for 6 rats), indicating that the carbon skeleton of the ^{14}C-carboxyl end was intact. Rats with a thoracic duct fistula excreted about 21—36 % of the

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** Approximately calculated as the difference between the fed activity and the activity excreted in the feces.

absorbed activity in the urine, while 39—64 % was found in the lymph lipids. Thus the acid was absorbed to a considerable extent *via* the portal vessels. 11—20 % of the lymph activity was present as free acid, 77—86 % as neutral fat, while only 2—5 % was incorporated into the phospholipids. A similar distribution in the lymph has been found for 2,2-dimethylstearic acid [2] and for 2,2,17,17-tetramethylstearic acid [3].

The active substances in the urine have been identified by reversed-phase partition chromatography and repeated recrystallizations with synthetic, unlabelled compounds. Two of the urinary metabolic products of 2,2-dimethylnonadecanoic acid were shown to be 2,2-dimethylglutaric (about 55 %) and 2,2-dimethylpimelic acid (about 25 %). Approximately 5 % of these acids were found in conjugated form. There was at least one other minor component, the composition of which has not yet been established. The metabolic products identified indicate that the two methyl groups completely block the degradation from the carboxyl end of the 2,2-dimethylnonadecanoic acid. As the degradation from 2,2-dimethylpimelic acid to 2,2-dimethylglutaric acid was not complete, the last step of the oxidation from the ω end was also influenced by the methyl groups. The steric hindrance thus extends its effect as far as the ∂ carbon atom. There was relatively more 2,2-dimethylpimelic acid in the urine on the first day after the administration than on the following days (Table 5).

Using fatty acids with a phenyl group at the β-carbon atom for blocking the β-oxidation Stevens [4] found that β-phenyldecanoic acid and β-phenyldodecanoic acid were excreted in the urine as β-phenyladipic acid. After feeding acids with an odd number of carbon atoms in the chain no homogeneous product could be isolated from the urine. However, it was clear that dibasic acids were excreted. It is to be noted that substituted acids with less than nine carbon atoms in the chain were excreted mainly unaltered in the urine. Yamakawa [5] administered 2-propyltridecanoic acid to a rabbit. As judged from the mixed melting points of bis-*p*-phenylphenacyl esters the urinary excretion products were suggested to consist of 2-propylglutaric acid (7 parts), and 2-propylpimelic acid (3 parts). Our results confirm these findings.

SYNTHESES

2,2-Dimethyl[1-^{14}C]nonadecanoic acid. A methanol solution (130 ml) of heptadecanoic acid (5.7 g), 3-methoxy[^{14}C]carbonyl-3-methylbutyric acid (0.5 g) and sodium (0.06 g) was electrolysed between Pt-electrodes with a current density of 0.3 A/cm^2 until the pH was 7 (*cf.* Ställberg-Stenhagen [6]). The solution was cooled to room temperature, and the *n*-dotriacontane formed was filtered off. It was extracted twice with boiling methanol and each time filtered off after cooling to room temperature. The combined methanol solutions were evaporated to dryness. The residue was extracted with ether repeatedly. The mixture obtained from the ether extract after evaporation of the solvent was chromatographed on acid alumina (activated at 180° overnight). The hydrocarbons formed were eluted with light petroleum, the methyl 2,2-dimethyl[1-^{14}C]nonadecanoate with benzene, whereas the acids, which had not reacted, and the alcohols formed as byproducts were left on the column.

The colourless crystals of methyl 2,2-dimethyl[1-^{14}C]nonadecanoate (0.5 g) were hydrolysed with a solution of potassium hydroxide (3 g) in water (2 ml) and ethanol (23 ml) for 5 hours in an autoclave at 120°. Water was added to the hydrolysate, which was acidified and extracted twice with ether. The ether solution was washed with water and dried with sodium sulphate, and the ether was evaporated. After recrystallization

from methanol and light petroleum the 2,2-dimethyl[1-^{14}C]nonadecanoic acid melted at 67.2–67.7°. The yield was 0.35 g. (Found: C 77.3; H 13.0; equiv. wt. 326. Calc. for $C_{21}H_{42}O_2$: C 77.2; H 13.0; equiv. wt. 326.5).

2,2-Dimethylglutaric acid. The synthesis of 2,2-dimethylglutaric acid has previously been reported by several investigators. One of the better methods was that described by Franke and Bueren [7], who obtained the acid in a 75 % yield from 4-cyano-2,2-dimethyl-1-butyraldehyde (starting material: 2-methylpropionaldehyde and vinylcyanide).

In the present investigation it was synthesized by a Kolbe electrolysis from methyl hydrogen malonate (4.8 g), 3-methoxycarbonyl-3-methyl-butyric acid (1.6 g) and sodium (0.1 g) in methanol solution (30 ml). The electrolysis was continued until the pH was 7. The methanol was evaporated and the ether-soluble fraction was isolated. The excess methyl hydrogen malonate was removed from the ether solution by the ion exchanger Amberlite IRA-400. The esters (2.7 g of crude product) were partially hydrolysed with potassium hydroxide (3 g), water (2 ml) and ethanol (30 ml) at room temperature, when the hindered ester groups were almost unattacked. After the addition of water (50 ml) the solution was extracted twice with ether to remove methyl tetramethyladipate. The water-ethanol layer was acidified and extracted three times with ether. The ether solution was dried with sodium sulphate, and the solvents were evaporated. Extraction of the residue with ether left most of the succinic acid undissolved. After removal of the ether, the esters were hydrolysed with potassium hydroxide (3 g), water (2 ml) and ethanol (23 ml) in an autoclave at 120° for 5 hours. Water (18 ml) was added to the solution, which was then acidified, saturated with ammonium sulphate and extracted with ether 5 times. The ether solution was dried with sodium sulphate and evaporated to dryness. The mixture of acids obtained was chromatographed twice on hydrophobic Hyflo supercel with 2-ethylhexanol-chloroform (6:5, v/v) as the stationary phase and methanol-water (2:3, v/v) as the moving phase. They were eluted in the order: succinic, dimethylglutaric, tetramethyladipic acid.

The 2,2-dimethylglutaric acid (0.5 g) was recrystallized from concentrated hydrochloric acid and from benzene-light petroleum; m. p. 82°.

2,2-Dimethylpimelic acid. Methyl hydrogen glutarate (7.3 g), 3-methoxycarbonyl-3-methylbutyric acid (1.7 g) and sodium (0.1 g) were electrolysed in methanol (75 ml) as described above. A great excess of glutarate was used in order to suppress the formation of 2,2,5,5-tetramethyladipic acid.

The solution was hydrolysed with potassium hydroxide (8 g) and water (5 ml) in an autoclave at 120° for 3 hours. After acidification the hydrolysate was extracted with ether. From the dry substance of the ether extract, chloroform dissolved the dimethylpimelic and tetramethyladipic acids but left a considerable amount of the glutaric and suberic acid undissolved. The chloroform extract was evaporated to dryness and the solid residue was chromatographed twice on hydrophobic Hyflo supercel with 2-ethylhexanol-chloroform (1:1, v/v) as the stationary phase and methanol-water (2:3, v/v) as the moving phase. The acids appeared in the following order: glutaric, suberic, dimethylpimelic and tetramethyladipic acid.

The 2,2-dimethylpimelic acid (0.66 g) was recrystallized from water and from benzene-light petroleum. M. p. 75.4°. (Found: C 57.6; H 8.4. Calc. for $C_9H_{16}O_4$: C 57.4; H 8.6.)

METABOLIC EXPERIMENTS

A stock solution of 2,2-dimethyl[1-^{14}C]nonadecanoic acid (241.2 mg) with a specific activity of 125 000 counts/min/mg in olive oil (12.3 g) was prepared, and 0.75 — 1 ml of this was given orally in a single dose to adult male rats weighing about 250 g. The radioactivity was determined with a windowless GM-tube (with a background effect of 40 c.p.m.) after direct plating on aluminium planchets. For further experimental details see Bergström, Borgström, Tryding and Westöö [1].

Isotope recovery in excreta after the administration of 2,2-dimethyl[1-^{14}C]nonadecanoic acid

A rat was fed 0.75 ml of the above-mentioned stock solution (1 600 000 counts/min). Feces from the first 24 hours after the administration were shown to contain 17 % of the fed activity. From Table 1 it is seen that the total activity recovered in the feces in one week was 22 % of the amount administered. The total activity found in the urine during one week after the administration, 70 %, corresponds to a recovery of 90 % of the absorbed activity.

The feces from five other rats were collected for 4 days after the administration of the active acid. They contained 9—34 % of the fed activity. In the same experiment 70—91 % of the absorbed activity could be recovered from the urine during the same time.

When part of an ether extract from hydrolysed feces containing 34 % of the fed activity was chromatographed according to Howard and Martin [8] (75 % aqueous acetone as the moving phase and paraffin oil as the stationary phase supported on hydrophobic kieselguhr), 92 % of the activity was found as unaltered 2,2-dimethylnonadecanoic acid. Another part of the same extract was chromatographed on hydrophobic kieselguhr (40 % methanol as the moving phase and 2-ethylhexanol-chloroform (1:1) as the stationary phase) [1]. In this way 3 % of the active substances could be identified as 2,2-dimethylpimelic acid and 2.5 % as 2,2-dimethylglutaric acid. 2.5 %, which was eluted with the front in this system, has not yet been identified. These dicarboxylic acids are real fecal metabolites. They cannot originate from the urine, which for this special animal has been collected separately *via* a plastic funnel, sewn on to the cutis around the penis of the rat. The reason for the presence of dicarboxylic acids in the feces is being investigated.

Table 1. Activity recovered in rat urine and feces after oral administration of 2,2-dimethyl-[1-^{14}C]nonadecanoic acid.

Day No.	Per cent of administered activity found in	
	feces	urine
1	17.4	60.3
2	3.9	6.1
3—4	0.6	3.1
5—7	0.1	0.3

Administration of 2,2-dimethyl[1-^{14}C]nonadecanoic acid to rat with a lymph fistula

Rats with a thoracic duct cannula (Bergström, Blomstrand and Borgström [9]) were fed the stock solution. The lymph was collected at 0° (Table 2, rats 1—3) or in ethanol (Table 2, rats 4—6). During the first 12 hours 52 %

Table 2. Recovery of isotope in lymph and urine after feeding 2,2-dimethylnonadecanoic acid.

Rat No.	Per cent of fed activity absorbed	Per cent of absorbed activity recovered in lymph lipids	Per cent of lymph activity recovered as			Per cent of absorbed activity recovered in urine
			glycerides	free acids	phospholipids	
1	88.2	63.6	86.3	11.1	2.6	29.0
2	66.8	54.0	78.1	19.8	2.1	35.1
3	80.2	55.3	77.2	17.8	5.0	28.7
4	92.5	47.4	82.3	15.9	1.8	35.6
5	74.1	39.5				36.3
6	76.5	52.3				20.6

of the fed activity was recovered in the lymph from rat No. 1 (Table 2). Another 4 % was found during the next 24 hours. Accordingly 24-hour-specimens were collected from the other fistula rats (Table 2). The total fat from the lymph samples was separated into a neutral fat-fatty acid fraction and a phospholipid fraction on a column of silicic acid (Borgström [10]). The phospholipids contained 2—5 % of the activity of the lymph lipids. The neutral fat was separated from free acids on the ion exchanger Amberlite IRA-400 (Borgström [11]). The free fatty acid fraction was shown to contain 11—20 % of the lymph activity.

In addition the excreta from these rats were collected. In four days 8—33 % of the administered activity was found in the feces. During the same period 21—36 % of the absorbed activity was recovered from the urine, while 39—64 % of the absorbed isotope was in the lymph.

Identification of isotopic compounds in the urine after the administration of 2,2-dimethyl[1-^{14}C]nonadecanoic acid

The urine from a noncannulated rat was acidified with hydrochloric acid. When the urine from the first day was extracted three times with two volumes of ether, 77 % of the activity was found in the ether phase. In the same way 60 % of the activity in the urine from the second day and 40 % of the activity from the third day could be extracted with ether. Portions of the urine fractions with a weight of substance less than 50 mg were examined by reversed-phase partition chromatography. The phases and procedures were the same as described earlier [1]. The chromatogram of the ether extract from the first-day urine showed three distinct active bands (*cf.* Fig. 1).

Approximately 15 % (30 mg) of the residue of the ether extract was mixed with inactive 2,2-dimethylglutaric acid (8.4 mg) and subjected to partition chromatography. It is shown in Fig. 1 that this acid corresponds to the second active band. (The front titration peak represents ordinary urine excretion products.) The third active band was rechromatographed together with 7.6 mg

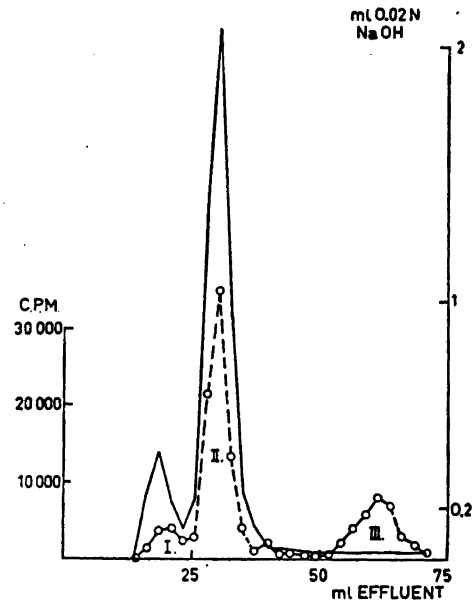

Fig. 1. Partition chromatography of 15 % of the ether extract from the first-day urine together with unlabelled 2,2-dimethylglutaric acid (8.4 mg). Stationary phase: 4 ml of i-octanol (2-ethylhexanol)-chloroform (1:1, v/v) supported on 4.5 g of hydrophobic Supercel. Moving phase: 40 % (v/v) aqueous methanol.

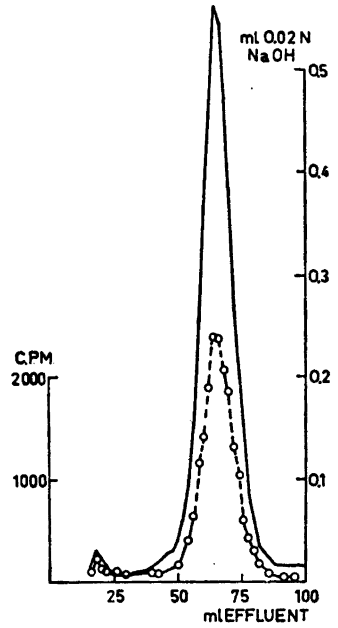

Fig. 2. Chromatography of the third active band from Fig. 1 with unlabelled 2,2-dimethylpimelic acid (7.6 mg). Phases: see Fig. 1.

Table 3. Specific activity of the most active fraction (1.2 mg) from the active band (II) of a chromatogram of urinary extract after recrystallization with inactive 2,2-dimethylglutaric acid (49.7 mg).

Recrystallization from	Crystal weight, mg	Counts / min / mg
benzene-light petroleum	41.5	2 225
water	26.0	2 313
water	14.3	2 280
benzene-light petroleum	9.9	2 307

Table 4. Specific activity of the two most active fractions (1.9 mg) from the active band III of a chromatogram of urinary extract after recrystallization with inactive 2,2-dimethylpimelic acid (79.0 mg).

Recrystallization from	Crystal weight, mg	Counts / min / mg
benzene-light petroleum	59.0	446
water	42.5	470
water	30.1	478
benzene-light petroleum	20.0	485
water	12.8	482

of 2,2-dimethylpimelic acid and the activity and titration peaks were found at the same position (Fig. 2).

The ether extract of the urine from a rat with a lymph fistula was subjected to reversed-phase partition chromatography. The isotope content of each fraction (2 ml) was determined after plating of 0.1 ml samples. The active bands appeared as in Fig. 1.

Table 5. The ratio between the three active bands in chromatograms of urine extracts.

Urine from day No.	Per cent of activity extracted with				Per cent of activity of band No.		
	ether		ethyl acetate from urine saturated with ammonium sulphate		I Conjugates with II and III. A small amount of an unidentified product	II	III
		after hydrolysis		after hydrolysis			
1			90		16	56	28
1				>90	13	58	29
1	80				9	61	30
1		80			8	61	31
2	60				34	55	11
3	40				40	51	9

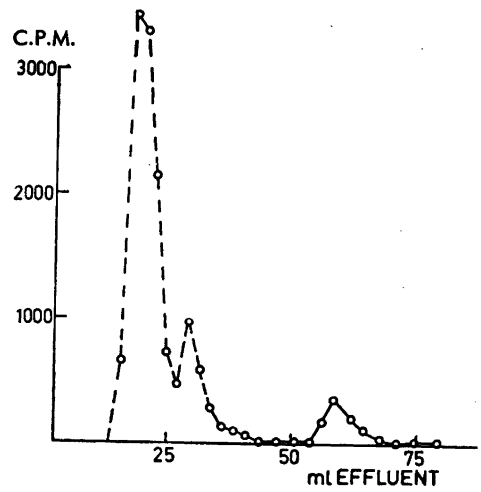

Fig. 3. Chromatogram of metabolites excreted in the urine, extracted with ethyl acetate after extraction with ether and saturation with ammonium sulphate. Phases: see Fig. 1.

The most active fraction from the second band with a weight of substance of 1.2 mg was mixed with 49.7 mg of inactive 2,2-dimethylglutaric acid and crystallized from benzene-light petroleum (b. p. 40—60°) and from water. As shown in Table 3 the activity remained constant indicating that 2,2-dimethylglutaric acid was excreted in the urine. In the same way recrystallization was performed with the two most active fractions from the third active band (with a dry weight of 1.9 mg) and inactive 2,2-dimethylpimelic acid

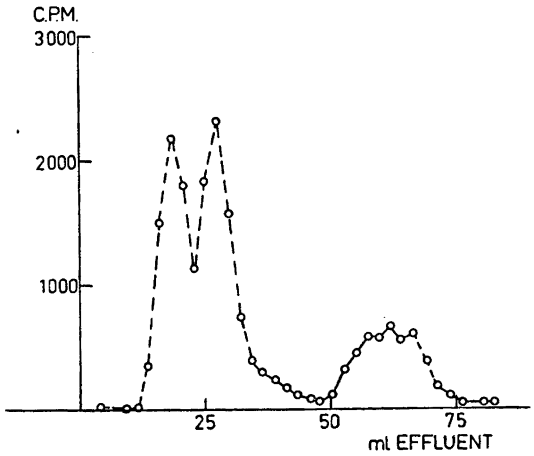

Fig. 4. Chromatogram of metabolites, after hydrolysis extracted as in Fig. 3. Phases: see Fig. 1.

(79.0 mg). From Table 4 it is seen that the activity remained constant. Consequently, 2,2-dimethylpimelic acid was another urinary metabolic product of 2,2-dimethylnonadecanoic acid.

As shown in Table 5 most of the activity of the ether extract was found in the second band (2,2-dimethylglutaric acid). In the chromatograms of the ether extracts of the urine from the second and the third day there was relatively more activity in the first band (unidentified product). The reverse was true for the third band (2,2-dimethylpimelic acid) which was distinctly lowered by the time. The ratio between the three active bands was almost unaltered after hydrolysis of the ether phase from the first-day urine. This indicates that no conjugates were present in the ether extract.

After the extraction with ether, half the water phase was saturated with ammonium sulphate and extracted three times with two volumes of ethyl acetate. In this way 47 % of the remaining activity could be extracted. When subjected to partition chromatography the main part of the activity was in the front band (Fig. 3). The other half of the water phase was hydrolysed for 6 hours at 120° with potassium hydroxide (4 g), water (2 ml) and ethanol (25 ml). After evaporation to dryness, the substance was dissolved in water, acidified, saturated with ammonium sulphate and extracted with ethyl acetate as described above. The activity could be extracted to 70 %.

A comparison between the chromatograms of ethyl acetate extracts of unhydrolysed and hydrolysed water phase of the ether-extracted urine from the first day (Figs. 3 and 4) shows that the front active band contains conjugates with the components in the second and third band. However, all the active substances in the first band could not be hydrolysed, which indicates the existence of a third metabolic product. This product is still being investigated.

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