BIOLOGICAL ACTIVITIES OF OPTICAL ISOMERS OF 6-CHLORO-5-CYCLOHEXYLINDAN-1-CARBOXYLIC ACID (TAI-284: ANTI-INFLAMMATORY AGENT)

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Abstract--A potent anti-inflammatory compound, TAI-284, and its optical isomers were subjected to the pharmacological, toxicological and metabolic studies in rats, mice and guinea-pigs. In anti-inflammatory, analgesic and antipyretic activities examined in rats and mice, the d-isomer was the most potent, followed by the racemate, and the l-isomer was the lowest. Similar results were obtained regarding toxicity and ulcerogenicity. The d-isomer maintained the highest plasma level, followed in decreasing order by the racemate and the l-isomer, when they were administered orally to rats. In the same experiment, the plasma level of the l-isomer's metabolites was the highest, followed in decreasing order by that of the racemate's metabolites and that of the d-isomer's metabolites. In Vitro, the l-isomer was most rapidly transformed into the metabolites while the d-isomer was most slowly transformed. On the other hand, the d-isomer was as potent as the l-isomer in the anti-inflammatory activity in guinea-pigs. In this species TAI-284 is hardly biotransformed into the metabolites. It is postulated that the rate of biotransformation of these compounds has a great influence on their pharmacological activities.

Recently several non-steroidal anti-inflammatory compounds bearing an asymmetric carbon have been synthesized, and their pharmacological activities have been reported. Some of the compounds revealed a difference in activity between the optical isomers (1, 2), while others did not (3, 4). 6-Chloro-5-cyclohexylindan-1-carboxylic acid (TAI-284) is a new non-steroidal compound which has strong anti-inflammatory, analgesic and antipyretic activities (5). This compound possessing an asymmetric carbon at C1 was resolved into the optical isomers by Noguchi et al. (6) as shown in Fig. 1.

Fig. 1. Chemical structures of enantiomers of TAI-284.
In preliminary studies, it was revealed that the $d$-isomer had a higher anti-inflammatory activity than did the $l$-isomer. Differences in intrinsic activity may be regarded as playing a major role in the mechanisms of activity. The present paper describes a comparative investigation of the racemate and optical isomers regarding anti-inflammatory, analgesic, antipyretic and toxic activities, as well as plasma levels and metabolic transformation.

MATERIALS AND METHODS

Male and female SD-JCL rats, male Hartley albino guinea-pigs and male ICR-JCL mice were used. Test agents were suspended with 4% gum acacia in water and were administered p.o. in the volume of 1 ml per 100 g body weight to rats and guinea-pigs, and 0.2 ml per 10 g body weight to mice. For i.v. administration to rats, the test agents were dissolved in physiological saline in the form of sodium salt and administered in 0.5 ml per 100 g body weight. The experiments were carried out following a double blind schedule.

1. Anti-inflammatory tests

1. Carrageenin edema

Male rats 6 weeks old, weighing 190 ± 10 g were used. Following the method of Winter et al. (7), the basic volume of a right hind paw was estimated. Drugs were administered 1 ml per 100 g body weight, followed immediately by an oral administration of water to a total of 5 ml per rat. One hr later the paw was injected s.c. with 0.05 ml of 1% carrageenin suspension in physiological saline to induce an edema. For drug testing, increase in volume of the foot 3 hr after phlogistic agent was adopted as a measure of effect.

2. Granuloma pouch

Female rats 6.5 weeks old, weighing 170 ± 10 g were used. Following the method of Robert and Nezamis (8), the rats were anesthetized with an i.p. injection of 75 mg/kg of sodium methylhexabital and 25 ml of air was injected s.c. at a clipped area on the back of the animal to make a pouch. Half ml of 1% croton oil solution in arachis oil was injected into the pouch. The test agents were administered daily for 5 days from the day of the pouch formation. The air in the pouch was withdrawn 48 hr after the croton oil injection. Immediately thereafter and again 24 hr later, the pouch area was gently massaged to prevent intra-pouch adhesion and promote an accumulation of exudate. The animals were sacrificed on the 6th day and the exudate volume was estimated for an evaluation of anti-inflammatory activity of test agents. In addition the weight of thymus, adrenal and spleen was estimated.

3. Ultraviolet erythema

Following the procedure of Winder et al. using a Hanovia Analytical Model Quartz Lamp (9), guinea-pigs weighing about 350 g were subjected to an ultraviolet irradiation on the depilated flank. Test agents were administered one hr before and immediately after the irradiation. Two hr after irradiation a degree of erythema was scored. An exposed spot with no evident erythema was scored 0, and the spot with a full circle of definite redness scored 1. A spot with erythema but not a complete circle of it was scored 0.5. Animals with total 3-spot scores of 2.0 to 3.0 were judged to be erythemic, those
with 1.5 or less as protected from erythema.

II. Analgesic test (Phenylquinone writhing test)

Following Siegmund et al. (10), mice 3.5 weeks old, weighing 18±2 g were injected i.p. with an aqueous solution of 0.02% phenylquinone (dissolved by adding 5% ethanol) in the volume of 0.1 ml per 10 g body weight 30 min after an administration of test agents. For 20 min after the phenylquinone injection the frequency of writhing or stretching responses was counted. The analgesic activity was taken as the inhibitory rate of these responses.

III. Antipyretic test

Male rats 6.5 weeks old, weighing 210±10 g were made febrile, following the method of Winder et al. (11), by a s.c. injection of the 15% baker’s yeast suspension in physiological saline in a volume of 1 ml per 100 g body weight. The animals were then fasted until the termination of experiment, expect for drinking water which was given ad libitum until estimation of body temperature was started. Rectal temperature was estimated every hour from 16 hr after injection of the yeast. The first three estimations were followed by administration of test agents. Estimation of the temperature was carried out for another 5 hours.

IV. Toxicology

1. Acute toxicity

Male rats 4.5 weeks old, weighing 140±10 g were used for tests of acute toxicity. After administration of test agents, behavior and body weight gain were examined for 7 days. An approximate LD50 was obtained from the mortality on the final day. All animals living and dead were autopsied.

2. Gastro-intestinal ulcers

Male rats 5 weeks old, weighing 150±10 g were administered 15 and 30 mg/kg of the test agents. These doses of TAI-284 were previously confirmed to produce G.I. ulcers in rats (12). The digestive tract from stomach to colon was removed 6 hr after administration. Mucosal ulcers were examined regarding number and area.

V. Metabolism

1. Oral administration

Male rats 5 weeks old, weighing 150±10 g were anaesthetized with ether at 2, 6 and 12 hr after an administration of 10 mg/kg of test agents. Blood samples were withdrawn from the inferior vena cava into a syringe containing heparin and were immediately centrifuged to obtain plasma.

2. Intravenous administration

A sodium salt solution of test agents in a dose of 1 mg/kg was administered i.v. to male rats. Blood was sampled at 20, 60 and 120 min after administration and plasma was obtained as described above.

Quantitative determination of TAI-284 and the metabolites in plasma: One ml of sample plasma was placed in a centrifuge tube, and 3 ml of 1N HCL and 30 ml of n-hexane containing 2.5% isoamyl alcohol were added. The mixture was shaken for 15 min for
Gas chromatograms obtained from rat plasma following dose of TAI-284 and from rat liver homogenate incubated with TAI-284. The dotted line represents the control plasma or homogenate. TAI-DCE: TAI-284 2,2-dichloroethyl ester. T: Unidentified components resulting from the pretreatment procedure. P: Unidentified components in plasma or homogenate. M1: Metabolites of TAI-284 (Metabolite II, III and/or IV) (14). M2: Metabolite of TAI-284 (Metabolite I) (14). CDA-DCH: Chlorendic acid dicyclohexyl ester, internal standard.

Conditions for gas chromatography are as follows:
- Column: 0.5% OV-17 on Chromosorb G (HP, 80/100 mesh) in 2.5 mm i.d. x 2 m glass tube.
- Column temperature: 260°C.
- Carrier gas: N2, 1.8 kg/cm², 20 ml/min.
- Detector: ECD, 63 Ni, 15 mCi, applied voltage 10 V, pulse being fixed at 10 x 100 usec (width x period).
- Instrument: Yanaco G-800E.

Deproteinization and extraction of TAI-284, then centrifuged at 3,000 r.p.m. for 5 min. The upper layer was put into a separation funnel, and a small amount of residual n-hexane was diluted with 10 ml of n-hexane without shaking. The n-hexane dilute was put together with the upper layer and extracted twice with 8 ml of 0.1 N NaOH by each 2 min shaking. The alkaline layer was separated and acidified by means of 0.5 ml of 5N HCL. The acid solution was extracted using each 5 ml and 3 ml of benzene successively with centrifugation. The benzene solution was collected and the solvent was evaporated off at 50°C under a stream of nitrogen. The residue was reacted with 100 μl of 2, 2-dichloroethanol and 100 μl of boron trifluoride ethyl ether complex at 70°C in a sealed tube for 45 min. The reaction mixture was extracted with 4 ml of n-hexane and washed twice with 10 ml of water to remove the excessive reagents, after which the solvent was evap-
porated off under a nitrogen stream at room temperature. The 2, 2-dichloroethyl ester of TAI-284 thus produced was determined with a gas chromatograph equipped with an electron capture detector (Fig. 2). The metabolites bearing a ketone or hydroxyl group on the cyclohexane ring of each parent compound were also determined simultaneously.

3. In Vitro test

Male rats 6 weeks old, weighing 160 ± 10 g were exsanguinated and 5 g of the liver was removed, homogenized with 0.2 M phosphate buffer and subjected to an ultracentrifugation at 9,000 g for 20 min. The resultant supernatant containing microsomes was isolated and diluted 32 times with the phosphate buffer. Two ml of this dilute was added to 3 ml of a mixture solution containing the test agent, triphosphopyridine nucleotide, glucose-6-phosphate, nicotinamide and MgSO₄, and thus their final concentrations were $10^{-5}$ M, $5.5 \times 10^{-5}$ M, $5 \times 10^{-3}$ M, $2 \times 10^{-2}$ M and $5 \times 10^{-3}$ M, respectively. The mixed solution was incubated following the method of McLuen et al. (13). The microsomal enzymes were inactivated by heating 20, 40 and 60 min after commencement of incubation.

Test agents and their metabolites were assayed by the procedure described above (Fig. 2). Metabolic transformation rate was estimated as the ratio of oxidized metabolites to the parent compound.

RESULTS

1. Anti-inflammatory activity

1. Carrageenin edema

As shown in Fig. 3, the anti-inflammatory activity of the d-isomer was somewhat higher than that of the racemate in a dose range from 1 to 3 mg/kg. On the other hand, the l-isomer was slightly effective at 6 mg/kg and significantly effective at 12 mg/kg. The dose-response lines of these compounds were, however, parallel.

![Fig. 3. Anti-inflammatory activity of TAI-284 and its enantiomers in carrageenin edema test in rats.](image-url)
2. Granuloma pouch

At the daily doses of 1 and 2 mg/kg, the d-isomer produced a higher anti-exudative activity than that of the racemate. The l-isomer was much less effective. These treatments did not affect the weights of thymus, adrenal and spleen (Table 1).

| Compound | Exp. No. | Oral dose mg/kg | No. of rats | Mean exudate volume ml ± S.E. | % Inhibit. | Mean organ weights |
|----------|----------|-----------------|-------------|-------------------------------|------------|--------------------|
| Control  | 1        | --              | 9           | 9.62 ± 0.69                   | 31.6       | 496                | 710                |
|          | 2        | 9               | --          | 8.66 ± 0.41                   | --         | 22.6               | 423                | 696                |
| dl-TAI-284 | 1      | 1.0             | 9           | 6.74 ± 0.48*                  | 29.9       | 29.7               | 548                | 784                |
|          | 2        | 1.0             | 9           | 6.89 ± 0.30*                  | 20.4       | 22.8               | 498                | 686                |
|          | 1        | 2.0             | 9           | 6.98 ± 0.23*                  | 27.4       | 30.4               | 500                | 737                |
| d-TAI-284 | 1      | 1.0             | 9           | 6.16 ± 0.48*                  | 35.8       | 29.6               | 466                | 707                |
|          | 2        | 1.0             | 9           | 6.54 ± 0.49b                  | 24.4       | 22.2               | 488                | 727                |
|          | 1        | 2.0             | 9           | 5.88 ± 0.54b                  | 38.9       | 28.8               | 506                | 766                |
| l-TAI-284 | 2      | 1.0             | 9           | 7.82 ± 0.37                   | 9.7        | 22.9               | 476                | 730                |
|          | 1        | 2.0             | 9           | 8.26 ± 0.53                   | 14.1       | 29.1               | 561                | 703                |

* : P<0.01

3. Ultraviolet erythema

As shown in Fig. 4, the racemate and d- and l-isomers revealed similar anti-erythemic activities in guinea-pigs. Thus the l-isomer, as well as the d-isomer, proved to be effective at 1 to 3 mg/kg.

II. Analgesic activity

The analgesic activity of the d-isomer examined by the phenylquinone writhing test was the highest followed by the racemate. The dose-response lines of these test agents

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**Fig. 4.** Anti-UV-erythemic activity of TAI-284 and its enantiomers in guinea-pigs.

**Fig. 5.** Analgesic activity of TAI-284 and its enantiomers in phenylquinone writhing syndrome test in mice.
were parallel, and the l-isomer showed much lower activity than that of the former two as shown in Fig. 5.

III. Antipyretic activity

As shown in Fig. 6, the d-isomer lowered the febrile body temperature of rats injected with baker's yeast more markedly than did the racemate. The l-isomer was much less effective. The d-isomer at 0.1 mg/kg was more potent than the l-isomer at 1.2 mg/kg, while antipyretic patterns appeared to be similar.

IV. Toxicology

1. Acute toxicity

In clinical and autopsic findings signs of toxicity were all related to lesions in the G.I. tract. The animals showing a lack of activity were anaemic in appearance, had a slow growth rate and ulcer formation was evident in the G.I. tract. Perforation of intestinal ulcers and consequent peritonitis led to the death of the animals. As to mortality and toxic findings, the d-isomer produced a somewhat higher toxicity than did the racemate as shown in Table 2. The approximate LD50 of the former was 30 mg/kg, while that of the latter was 40 mg/kg.

| Compound | Oral dose mg kg | B.W. loss | Bloody excrement | Stom. a | Ulcers | Jej. a | Heum | Adhes. a | Ascites | % Mortality |
|----------|----------------|-----------|-----------------|--------|--------|--------|------|----------|---------|-------------|
| dl- TAI-284 | 20     | 3/6       | 2/6             | 1.6    | 6/6    | 6/6    | 6/6  | 3/6      | 3/6     | 16.7        |
|           | 40     | 5/6       | 4/6             | 4/6    | 6/6    | 6/6    | 6/6  | 5/6      | 5/6     | 50.0        |
|           | 60     | 6/6       | 4/6             | 6/6    | 6/6    | 6/6    | 6/6  | 5/6      | 6/6     | 83.3        |
| d- TAI-284 | 20     | 4/6       | 3/6             | 2/6    | 6/6    | 6/6    | 6/6  | 5/6      | 4/6     | 33.3        |
|           | 40     | 6/6       | 6/6             | 4/6    | 6/6    | 6/6    | 6/6  | 6/6      | 6/6     | 100.0       |
|           | 60     | 6/6       | 5/6             | 6/6    | 6/6    | 6/6    | 6/6  | 6/6      | 6/6     | 100.0       |
| l- TAI-284 | 20     | 0/6       | 0/6             | 0/6    | 1/6    | 6/6    | 6/6  | 0/6      | 0/6     | 0           |
|           | 40     | 0/6       | 2/6             | 0/6    | 6/6    | 6/6    | 6/6  | 5/6      | 3/6     | 0           |
|           | 80     | 0/6       |                 |        |        |        |      |          |         |             |

a: Stomach; b: Jejunum; c: Adhesion
2. Gastro-intestinal ulcers

The racemate and two isomers produced ulcers similar in shape and distribution six hours after administration. These ulcers were found exclusively in the jejunum and ileum. Regarding ulcerogenicity the d-isomer proved to be somewhat stronger than the racemate and the l-isomer produced few ulcers as shown in Table 3.

| Compound    | Exp. No. | Oral dose mg/kg | No. of rats | Small-intestinal ulcers* | Mean ± S.E. | Number | Area (mm²) |
|-------------|----------|-----------------|-------------|--------------------------|-------------|--------|------------|
| d-TAI-284   | 1        | 15              | 6           | 91.5 ± 9.0               | 118.5 ± 14.9| 6      |            |
|             | 2        | 30              | 6           | 95.7 ± 10.6              | 119.3 ± 14.2| 6      |            |
| d-TAI-284   | 1        | 15              | 6           | 95.7 ± 6.8               | 120.3 ± 8.6 | 6      |            |
|             | 2        | 30              | 6           | 96.2 ± 6.9               | 121.8 ± 11.4| 6      |            |
| l-TAI-284   | 1        | 15              | 6           | 38.7 ± 8.2               | 38.7 ± 8.2  | 6      |            |
|             | 2        | 30              | 6           | 45.8 ± 8.9               | 45.8 ± 8.9  | 6      |            |

* : Examined 6 hr after administration.
* : P<0.01 as compared with dl-TAI-284.

V. Metabolism

1. Plasma level and metabolic transformation

Each plasma level curve of test agents after oral administration showed a peak at 2 hr, then declined in a manner which was similar. A significant difference seen among

![Plasma level of TAI-284 and its enantiomers after oral administration in rats.](image1)

![Plasma level of total metabolites of TAI-284 and its enantiomers after oral administration in rats.](image2)
them was that the d-isomer showed the highest concentration followed by the racemate, while the l-isomer was the lowest (Fig. 7). The plasma level of the d-isomer 2 hr after administration was 10.5 μg/ml, the racemate 7 μg/ml and the l-isomer 3.7 μg/ml. On the other hand, the plasma level of the metabolites of the test agents showed a reverse order. Thus, the metabolites of the l-isomer have the highest plasma level followed by those of the racemate and the d-isomer respectively (Fig. 8).

To exclude a possible absorptive factor in oral administration, plasma levels of test agents and their metabolites were estimated after intravenous administration, and the results were similar. The d-isomer maintained the highest plasma level, while its metabolites showed the lowest level (Fig. 9, 10).

2. Metabolism in rat liver homogenate

Similarly to the results in vivo, the biotransformation rate in vitro showed a significant difference among the three. The
l-isomer was most rapidly transformed into metabolites while the transforming rate of the d-isomer was the lowest as shown in Fig. 11.

DISCUSSION

TAI-284 was previously confirmed to possess potent anti-inflammatory, analgesic and antipyretic activities (5). In the present study, a marked difference in therapeutic and toxic activities was observed between the optical isomers of TAI-284 in rats and mice. The d-isomer showed a higher activity than the racemate against carrageenin edema of an acute inflammation and against exudation of a subchronic type. The l-isomer also possessed an anti-inflammatory effect, though much less potent than that of the d-isomer.

As for analgesic activity, the d-isomer was also the highest, followed by the racemate, while the l-isomer was the lowest. Thus in mice, as well as in rats, the difference in pharmacological activity was observed.

TAI-284 was previously confirmed to have an extremely strong antipyretic action. It was effective at the oral dose of 98 μg/kg in febrile rats and showed 5.5 times higher potency than indomethacin (5). In the present study, a comparison of antipyretic potency of the three compounds demonstrated that the d-isomer was approximately twice as potent as the racemate, while the l-isomer had one fifth the potency of the racemate.

Pharmacological properties of the optical isomers proved to be essentially similar, since their dose-response lines showed a parallelism. The anti-inflammatory, analgesic and antipyretic activities of TAI-284 are thus considered attributable to its d-component.

The d-isomer showed the highest acute toxicity and ulcerogenicity followed by the racemate, and the l-isomer had much lower activities. Therefore the order in such toxic activities of test agents showed a good accordance with that in their pharmacological activities described above. The shape and size of the ulcers and the distribution pattern were also found to be similar.

In order to investigate the mechanism for such a difference in biological activities, plasma level of test agents was estimated after an oral or intravenous administration to rats. The d-isomer was found to have the highest plasma level followed by the racemate, while the l-isomer had the lowest level. On the contrary, the biotransformation rate of the l-isomer determined by the plasma level of its metabolites was highest, while that of the d-isomer was lowest. Such a metabolic difference was clarified also by an in vitro experiment using rat liver homogenate. These results indicate that in rats and mice the biological activities of the three agents greatly depend on the metabolic inactivation. In this respect, it is interesting that the two isomers revealed equipotent anti-erythemic activity in guinea-pigs in which TAI-284 was hardly biotransformed as previously confirmed by Tanayama et al. (14). Schmid et al. found the d-isomer of α-phenyl-α-ethyl-glutarimide to be stronger than the l-isomer in sedative action (15). These authors reported the difference in plasma and tissue levels of the isomers which was brought about by inactivation in the liver. Furner et al. also suggested the same mechanism as described above, for a differential anesthetic activity of optical isomers of hexobarbital (16).
Several metabolites of TAI-284 were obtained by an infusion through rat liver and identified by Kanai et al. (17). Most of these bear a ketone or hydroxyl group on the cyclohexane ring of TAI-284, and most of these metabolites synthesized chemically by Kishimoto et al. proved to have lower pharmacological and toxic activities than those of the parent compound, though some of the metabolites had characteristic properties (18). Although the possibility of differences in intrinsic activity (6, 19), absorption and distribution (20) cannot be excluded, the activity difference among the d-isomer, the racemate and the l-isomer of TAI-284 is likely to be produced by the difference in their plasma levels which probably depends on a biotransformation in liver. This mechanism may be explained by “stereo-selective metabolism of enantiomers” as described by Furner et al. (16) and Brooks et al. (21).

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REFERENCES
1) Vincent, M., Remond, G. and Poignant, J.: J. med. Chem. 15, 75 (1972)
2) Harrison, I.T., Lewis, B., Nelson, P., Rooks, W., Roszkowski, A., Tomoloni, A. and Fried, J.H.: J. med. Chem. 13, 203 (1970)
3) Adams, S.S., Clift, E.E., Lesell, B. and Nicholson, J.S.: J. Pharm. Sci. 56, 1686 (1967)
4) Nickander, R.C., Kraay, R.J. and Marshall, W.S.: Fedn. Proc. 30, 563 (1971)
5) Kawai, K., Kuzuna, S., Morimoto, S., Ishii, H., and Matsumoto, N.: Japan. J. Pharmacol. 21, 621 (1971)
6) Noguchi, S., Kishimoto, S., Minamida, I., Obayashi, M. and Kawakita, K.: Chem. Pharm. Bull. 19, 646 (1971)
7) Winter, C.A., Risley, E.A. and Nuss, G.W.: Proc. Soc. exp. Biol. Med. 111, 544 (1962)
8) Robert, A. and Nizamis, J.E.: Acta Endocr. 25, 105 (1957)
9) Winder, C.V., Wax, J., Burr, V., Been, M. and Rosier, E.C.: Archs int. Pharmacodyn. Ther. 116, 261 (1958)
10) Siegmund, E., Cadmus, R. and Lu, G.: Proc. Soc. exp. Biol. Med. 95, 729 (1957)
11) Winder, C.V., Wax, J., Scotti, L., Scherrer, R.A., Jones, E.M. and Short, F.W.: J. Pharmacol. exp. Ther. 138, 405 (1963)
12) Kawai, K., Kanno, M., Kuzuna, S., Nomura, M., Maki, Y. and Matsumoto, N.: Folia pharmacol. japon. 67, 173p (1971) (in Japanese)
13) McLuen, E.F. and Fouts, J.R.: J. Pharmacol. exp. Ther. 131, 7 (1960)
14) Tanayama, S.: Xenobiotica, 3, 671 (1973)
15) Schmid, K., Riess, W. and Kieferle, H.: Isotopes in Experimental Pharmacology. Edited by Roth, L.L., p. 383, The University of Chicago Press, Chicago and London (1965)
16) Furner, R.L., McCarthy, J.S., Stitzel, R.E. and Anders, M.W.: J. Pharmacol. exp. Ther. 169, 153 (1969)
17) Kanai, Y., Kobayashi, T. and Tanayama, S.: Xenobiotica, 3, 657 (1973)
18) Kuzuna, S., Matsumoto, N. and Kawai, K.: Japan. J. Pharmacol. 24, 687 (1974)
19) Berkowitz, B.A. and Leong, W.E.: J. Pharmacol. exp. Ther. 177, 500 (1971)
20) Shindo, H., Nakajima, E., Komai, T., Tanaka, K., Miyakoshi, N. and Kawai, K.: Presented at the third Symposium on Drug Metabolism and Action, Fukuoka, Japan (1971) (in Japanese)
21) Brooks, G.T., Lewis, S.E. and Harrison, A.: Nature 220, 1034 (1968)