PROCEEDINGS OF THE ROYAL SOCIETY OF LONDON

SERIES B VOLUME 182
BIOLOGICAL SCIENCES

1972

PUBLISHED BY THE ROYAL SOCIETY
6 CARLTON HOUSE TERRACE LONDON SW1Y 5AG

The four numbers in this volume can be obtained separately from the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

Printed in Great Britain at the University Printing House, Cambridge

Proc. R. Soc. Lond. B. **182**, 25–35 (1972)
Printed in Great Britain

The conjugation of phenylacetic acid in man, sub-human primates and some non-primate species

By Margaret O. James, R. L. Smith, R. T. Williams, F.R.S. and M. Reidenberg

Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2

(*Received* 20 *March* 1972)

^{14}C-Labelled phenylacetic acid has been administered to man, 14 species of sub-human primates and 11 non-primate species and their urine examined for metabolites. Four amino acid conjugates of this acid have been found in various species, namely, phenacetylglutamine, phenacetylglycine, phenacetyltaurine and diphenacetylornithine, but their occurrence varies with species. In the primates the occurrence of the glutamine and glycine conjugates appears to be correlated with their evolutionary status. Man excretes exclusively the glutamine conjugate, the Old World monkeys the glutamine conjugate and very small amounts of the glycine conjugate, the New World monkeys the glutamine conjugate and significant amounts of the glycine conjugate and the prosimians the glycine conjugate only. The non-primate mammalian species excrete the glycine conjugate and no glutamine conjugate. The two avian species examined also differed, since the pigeon excreted the glycine conjugate, whereas the domestic hen excreted mainly the ornithine conjugate with small amounts of the glycine conjugate. The conjugation of phenylacetic acid with taurine is reported for the first time. It occurs in all the species examined except the vampire bat and domestic hen, but its quantitative occurrence is haphazard amongst the species examined. It was found in significant amounts in the pigeon, ferret, bushbaby, capuchin monkey, squirrel monkey, mona monkey and baboon, but in minor amounts in other species.

Introduction

Thierfelder & Sherwin (1914, 1915) found that when phenylacetic acid was ingested by human beings it was excreted in the urine as phenacetylglutamine. In animals such as the dog, rabbit and horse, it had been shown (Salkowski 1877, 1884; see also Quick 1932) that phenylacetic acid behaved like benzoic acid and was excreted as the glycine conjugate, phenaceturic acid. For many years it appeared that conjugation with glutamine was peculiar to man and to phenylacetic acid since several other species including the rhesus monkey (Sherwin 1917) were shown to form phenaceturic acid. However, Power (1936) reported that the chimpanzee, like man, also formed phenacetylglutamine and later, glutamine conjugates of 3,4-dihydroxy-5-methoxyphenylacetic acid as a metabolite of mescaline (Harley-Mason & Laird 1959) and homoanisic acid (4-methoxyphenylacetic acid) and its metabolite, 4-hydroxyphenylacetic acid (Oakley & Seakins 1971), were found to be formed in man. Four species of African monkeys were briefly reported by Patel & Crawford (1963) to form a glutamine conjugate with indoleacetic acid, although such a conjugate was not formed in the ring-tailed lemur or bushbaby. These findings suggested that glutamine conjugation might be a reaction of arylacetic

acids in man and certain monkeys but not in lemurs. However, Drach & Howell (1968) found that diphenylmethoxyacetic acid which is a metabolite of the antihistamine drug, Diphenhydramine(Benadryl; 2-diphenylmethoxy-*N*,*N*-dimethylethylamine), but is not an arylacetic acid although closely related, formed a glutamine conjugate in the rhesus monkey. There was also other work published which suggested that the substituted phenylacetic acids, 4-chloro- and 4-bromophenylacetic acid, did not form glutamine but glycine conjugates in man (Cerecedo & Sherwin 1924).

It would appear from what has been said that there is much uncertainty about the species distribution of glutamine conjugation and the type of substituted acetic acid which is so conjugated. We have therefore examined the fate of phenylacetic acid and some of its 4-substituted derivatives and of indoleacetic acid in a number of primates and non-primates using the ^{14}C-labelled compounds. In this paper the findings with phenylacetic acid are described.

Materials and Methods

Compounds

Phenylacetic acid, m.p. 76 °C from water, was purchased and purified. [*carboxy*-^{14}C]Phenylacetic acid (377 μCi/mg) was supplied by the Radiochemical Centre, Amersham, U.K. Phenacetylglycine prepared according to Hotter (1888) had m.p. 142–143 °C after recrystallization from water. The mass spectrum of its methyl ester showed a peak at *m/e* 207 corresponding to the parent ion (methyl phenaceturate). L-(−)-Phenacetylglutamine was prepared according to Thierfelder & Sherwin (1914) and had m.p. 100–101 °C from ethyl acetate and showed $[\alpha]_D^{21} - 15.6°$ (c = 10 in water). The mass spectrum of its methyl ester showed a peak at *m/e* 278 corresponding to the parent ion.

L-(+)-Diphenacetylornithine has been prepared biosynthetically by Totani (1910). It was prepared synthetically by treating a solution of L-(+)-ornithine monohydrochloride (5 g) in water (150 ml) containing sodium bicarbonate (50 g) with phenacetyl chloride (15.2 g). The solution was then acidified with 10 M-HCl and an oil separated on standing. The oil was taken up in ether (100 ml) and the solution kept at room temperature. A white solid separated which was filtered and recrystallized from aqueous ethanol. The L-(+)-diphenacetylornithine (2.5 g) formed small white crystals, m.p. 140 °C and $[\alpha]_D^{25} + 9.5°$ (c = 6.5 in 0.1 M NaOH). Totani (1910) gives m.p. 139–139.5 °C for the biosynthetic compound. The mass spectrum of the product of methylation of the compound with diazomethane in ether gave a peak at *m/e* 382 corresponding to the molecular ion of diphenacetylornithine methyl ester.

Phenacetyltaurine has not been described previously. A solution of phenylacetic acid (3 g) in dry dioxan (10 ml) containing tri-*n*-butylamine (5.3 ml) was cooled to 5 °C and treated with ethyl chloroformate (2.5 ml). The product was kept at 5 °C for 30 min and then a solution of taurine (3.45 g) in M NaOH (27.5 ml) was

added. The mixture was kept for 30 min at room temperature and then evaporated almost to dryness at 40 °C under diminished pressure in a rotary evaporator. The residue was dissolved in 50 % aqueous methanol (5 ml), the solution brought to pH 10 with 2 M NaOH and extracted with light petroleum (b.p. 40–60 °C; 3 × 20 ml). The aqueous layer was separated, neutralized with 2 M HCl and evaporated nearly to dryness at 40 °C as before. The residue from this was now extracted with a 2:1 mixture of chloroform and methanol (20 ml). This extract was filtered and evaporated to near dryness to a residue which was now dissolved in 2 M HCl (3 ml). This solution was extracted with ether (3 × 10 ml) to remove unchanged phenylacetic acid and the separated aqueous layer allowed to go to dryness in a vacuum desiccator to form a glassy solid. The latter was dissolved in warm ethanol (100 ml), the solution filtered and then evaporated to about 5 ml. On keeping small white crystals separated (0.5 g). These had no sharp melting point but decomposed at about 250 °C. A sample (10 mg) in methanol (5 ml) was treated with diazomethane and a portion (2 μl) of the reaction mixture submitted to mass spectrometry. The mass spectrum gave a peak at *m/e* 257 which corresponded to the molecular ion of phenacetyltaurine methyl ester.

The crystalline material gave no ninhydrin reaction for free amino acid. However, it (10 mg) was heated at 110 °C for 2 h with 6 M HCl (2 ml). After cooling the solution was extracted with ether (3 × 5 ml) and the aqueous phase was separated and reduced to dryness at 40 °C on a rotary evaporator. The residue was taken up in water (1 ml) and portions (0.01 ml) of the solution chromatographed on Whatman no. 2 paper using two solvents separately, namely, butan-1-ol–pyridine–water (1:1:1 by vol) and phenol saturated with water. A ninhydrin positive spot appeared at R_F 0.35 in the first solvent and at R_F 0.39 in the second. These R_F values were identical with those of authentic taurine in these solvents.

Animals

The rhesus and cynomolgus monkeys, the New World monkeys, the prosimian species, the rodents, carnivores and birds were obtained from animal dealers in the London area. We are grateful to the University of Ibadan Zoo, Nigeria, for allowing us to use in Nigeria the green monkey, the red-bellied monkey, mona monkey, mangabey and drill and to the Nuffield Institute of Comparative Medicine, London, for access to a baboon and the vampire bats.

Phenyl[^{14}C]acetic acid was administered in water as the sodium salt and for injection into primates the solution was sterilized by filtration. Urine was collected for 24 h and analysed immediately after collection, except the samples which were brought by air from Nigeria, and in this case the urines were preserved with mercuric chloride.

Chromatography

The colour reactions and R_F values of phenylacetic acid and its conjugates are shown in table 1. Radiochromatogram scans of urine were prepared by placing the urine (0.01–0.2 ml containing about 2×10^4 d.p.m.) as a band on a strip (5 cm

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.