Metabolism of 3-Methyl-4-phenyl-3-butenamide

(Received for publication, December 6, 1967)

L. CANONICA, P. MANITTO, U. VALCAVI, AND N. ZONTA BOLEGO

From the Istituto Chimica Organica, Facoltà di Scienze, Università di Milano and Istituto Biochimico Italiano, Milan, Italy

SUMMARY

Urine of rabbits and rats treated with large doses of 3-methyl-4-phenyl-3-butenamide (I) was analyzed. Four metabolic products were isolated and identified: 2-hydroxy-3-methyl-4-phenyl-3-butenamide (II), 3-methyl-4-(4'-hydroxyphenyl)-3-butenamide (III), 3-benzyl-4-hydroxy-2-butenolic acid lactone (IV), and 3-(4'-hydroxybenzyl)-4-hydroxy-2-butenolic acid lactone (V). Compound IV was shown to be a metabolic intermediate between Compounds I and V. The metabolic fate of Compound I in man was found to be the same as in rat and rabbit.

3-Methyl-4-phenyl-3-butenamide (I) is a hypocholesteremic and hypolipemic agent, reducing, at least in part, the biosynthesis of cholesterol and fatty acids (5). This paper deals with the isolation, characterization, and synthesis of four metabolites of Compound I obtained from the urine of rats and rabbits receiving the drug.

EXPERIMENTAL PROCEDURE

Ultraviolet absorption spectra were determined in methanol on a Beckman DU spectrometer, model G 2400. Infrared spectra were recorded in Nujol on a Perkin-Elmer Infracord, model 137. Nuclear magnetic resonance spectra were recorded on a Perkin-Elmer R-10 (60 Mc) spectrometer, with deuterated pyridine as solvent and tetramethylsilane as internal reference; chemical shifts are reported in delta, parts per million (multiplicity, J, number of protons, and attributions are indicated in parentheses). Descending paper chromatography was performed on propylene glycol-methanol (1:3)-impregnated Whatman No. 1 paper, with benzene-water-methanol (2:2:1) as solvent and tetramethylsilane as internal reference; chemical shifts are reported in delta, parts per million (multiplicity, J, number of protons, and attributions are indicated in parentheses). Ascending paper chromatography was performed on Whatman No. 1 paper, with benzene-ethyl ether (1:1) impregnated Whatman No. 1 paper, with benzene-water-methanol (2:2:1) as solvent and tetramethylsilane as internal reference; chemical shifts are reported in delta, parts per million (multiplicity, J, number of protons, and attributions are indicated in parentheses). Descending paper chromatography was performed on Whatman No. 1 paper, with benzene-ethyl ether (1:2) impregnated Whatman No. 1 paper, with benzene-water-methanol (2:2:1) as solvent and tetramethylsilane as internal reference; chemical shifts are reported in delta, parts per million (multiplicity, J, number of protons, and attributions are indicated in parentheses).

Urine of rabbits and rats treated with large doses of 3-methyl-4-phenyl-3-butenamide (I) was analyzed. Four metabolic products were isolated and identified: 2-hydroxy-3-methyl-4-phenyl-3-butenamide (II), 3-methyl-4-(4'-hydroxyphenyl)-3-butenamide (III), 3-benzyl-4-hydroxy-2-butenolic acid lactone (IV), and 3-(4'-hydroxybenzyl)-4-hydroxy-2-butenolic acid lactone (V). Compound IV was shown to be a metabolic intermediate between Compounds I and V. The metabolic fate of Compound I in man was found to be the same as in rat and rabbit.
ethereal solution was washed with water and NaHC03, 5% solution, in Rabbit
urine of the next day, Compound V was identified by paper
chromatography. The same four metabolites were identified by paper
chromatography of Compound I, 0.9 g of Compound II, and 0.5 g of Compound
III. The same four metabolite compounds were also identified by paper chromatography in the bile of rat treated orally with 0.1 g of Compound I per kg.

Metabolism of 3-Methyl-4-phenyl-3-butenamide (Compound I) in Rabbit and Man—Ten male rabbits weighing 2.4 to 2.5 kg received orally 0.5 g of Compound I per kg daily for 10 days. Their pooled urine (10 liters) was worked up as reported above and afforded 1 g of Compound IV, 1.3 g of Compound V, 0.2 g of Compound I, 0.9 g of Compound II, and 0.5 g of Compound III. The same four metabolites were identified by paper chromatography in the urine of man treated orally with 0.01 g of Compound I per kg.

Metabolism of Compound IV to Compound V—Four male rats weighing 240 to 260 g received orally 0.5 g of Compound IV per kg for only 1 day. In the chloroform extract of their pooled urine of the next day, Compound V was identified by paper chromatography.

Synthesis of 2-Hydroxy-3-methyl-4-phenyl-3-butenamide (Compounds I and II)-3 4-Benzyl-4-hydroxy-2-butenolic acid lactone (Compound VII, R=H) (26.45 g) was treated in benzene (265 ml) and ethyl ether (265 ml) with zinc (52.9 g). After distillation of some solvent (53 ml), ethyl bromoacetate (66 ml) was added to the mixture, which was refluxed with stirring for 1 hour, treated with dioxane (150 ml), then refluxed again for 15 min, cooled, and finally deacetylated in a mixture of ethyl ether (560 ml) and hydrochloric acid 4% in water (560 ml). The etheral solution was washed with water, dried, and evaporated and the residue was refluxed for 48 hours with acetic anhydride (440 ml). Removing the solvent at 50°C and at 20 mm Hg of pressure, a residue was obtained which was extracted with ethyl ether. This etheral solution was washed with water and NaHCO3, 5%, in water, and evaporated to dryness. The residue obtained was dissolved in benzene and chromatographed on alumina (295 g). After 20 hours, the column was eluted with ethyl acetate (3 liters) and the solvent was evaporated. The crude material, distilled at 149°C and 0.1 mm Hg pressure, yielded 14.1 g of a product identical, by paper chromatography, ultraviolet and infrared spectra, and gas chromatography, with Compound IV obtained from urine as a metabolite of Compound I.

Metabolism of 3-Methyl-4-phenyl-3-butenamide (Compound I) and ethyl ether (265 ml) with zinc (52.9 g). After distillation of some solvent (53 ml), ethyl bromoacetate (66 ml) was added to the mixture, which was refluxed with stirring for 1 hour, treated with dioxane (150 ml), then refluxed again for 15 min, cooled, and finally deacetylated in a mixture of ethyl ether (560 ml) and hydrochloric acid 4% in water (560 ml). The etheral solution was washed with water, dried, and evaporated and the residue was refluxed for 48 hours with acetic anhydride (440 ml). Removing the solvent at 50°C and at 20 mm Hg of pressure, a residue was obtained which was extracted with ethyl ether. This etheral solution was washed with water and NaHCO3, 5%, in water, and evaporated to dryness. The residue obtained was dissolved in benzene and chromatographed on alumina (295 g). After 20 hours, the column was eluted with ethyl acetate (3 liters) and the solvent was evaporated. The crude material, distilled at 149°C and 0.1 mm Hg pressure, yielded 14.1 g of a product identical, by paper chromatography, ultraviolet and infrared spectra, and gas chromatography, with Compound IV obtained from urine as a metabolite of Compound I.

Synthesis of 3-(2-Hydroxybenzyloxy)-4-hydroxy-2-butenic Acid Lactone (Compound V)—4-Acetoxyphenylacetic acid chloride (Compound III, R=AcO—) (10.4 g), prepared according to Heilbron and Cook (9) and Heilborn, Cook, and Elvidge (10), was treated with diisomethane (obtained from 30 g of N-nitroso-N-methyleneurea) in methylene chloride (400 ml). After stirring the mixture for 2 hours at 20°C, the solvent was evaporated at room temperature and the residue was heated with acetic acid (100 ml) at 100°C for 30 min. After evaporation of the acetic acid at 50°C and dilution with water, the mixture was extracted with ethyl ether. The ethereal solution was washed with water, then with NaHCO3, 5%, in water, and with water again and finally evaporated to dryness. Crystallization of the residue from ethyl ether-hexane gave 1-acetoxy-3-(4'-acetoxyphenyl)-2-propanone (Compound VII, R=AcO—) (8.2 g), m.p. 50°C.

C19H19O5 (250.24)
Calculated: C 62.40, H 5.63
Found: C 62.66, H 5.67

The acetoxyketone (Compound VII, R=AcO—) (6 g) was treated with zinc and ethyl bromoacetate and then with acetic anhydride in the same conditions used for the synthesis of Compound IV. The crude product obtained, when chromatographed on alumina (67 g) eluting with ethyl ether-ethyl acetate (3:1), gave 0.5 g of a product shown to be identical, by paper chromatography, ultraviolet and infrared spectra, and melting point with Compound IV isolated from the urine as a metabolite of Compound I.

Synthesis of 2-Hydroxy-3-methyl-4-phenyl-3-butenic Acid Lactone (Compound II)—3-Methyl-4-phenyl-3-butenamide (Compound I) (90 g) was refluxed with stirring for 4 hours with dioxane (70 ml), water (2.2 ml), and selenium dioxide (13 g). After filtration of the mixture and evaporation of the solvent, the residue, when crystallized from ethyl acetate, afforded 3.9 g of a product whose ultraviolet and infrared spectra and melting point were identical with those of Compound II obtained from the urines as a metabolite of Compound I.

RESULTS
From the urine of rat and rabbit treated with 3-methyl-4-phenyl-3-butenamide (Compound I) four metabolic products have been isolated.

Structure I. Compound I

Metabolite II, C19H19O5N, was found to contain an amide group (infrared spectrum with absorption bands at 3350, 3200, and 1670 cm⁻¹), an olefinic bond conjugated with an aromatic ring (ultraviolet spectrum with absorption maximum at 245 nm), and a hydroxyl group (infrared spectrum with absorption band at 3450 cm⁻¹). The hydroxyl group is not present on the aromatic ring of Compound II (infrared spectrum with absorption bands at 760 and 705 cm⁻¹ characteristic of monosubstituted aromatics; no reaction in the Millon test). The nuclear magnetic
resonance spectrum of Compound II, similar to Compound I, shows a band at 2.20 δ (3H, CH=CH--C=CH--) but, instead of the band at 3.55 δ of Compound I, it shows a band at 5.12 δ corresponding to 1 proton. From these results the structure of 2-hydroxy-3-methyl-4-phenyl-3-butenamide was attributed to Metabolite II. This metabolite was found to be optically inactive and identical with the compound obtained by reaction of Compound I with selenium dioxide.

Structure II. Compound II

Metabolite III, C₁₁H₁₄O₂N, was found to contain an amide group (infrared spectrum with absorption bands at 3,300 and 1,670 cm⁻¹), an olefinic bond conjugated with an aromatic ring (ultraviolet spectrum with absorption maximum at 257 μm), and a hydroxyl group in the meta position of the benzene ring (positive reaction in the Millon test; infrared spectrum with absorption band at 3,600 cm⁻¹). Further support for Structure III was obtained from the nuclear magnetic resonance spectrum of this metabolite, showing signals at 3.65 δ (2H, -CH=CH--CONH-), 4.75 δ (2H, -O-CH=CH--), 5.90 δ (vinyl protons), 6.65 δ (phenolic proton), and 7.18 δ (3 aromatic protons) but no signal corresponding to a methyl group at 1.5 to 2.0 δ. The dihydroderivative of Metabolite V, obtained by hydrogenation of Metabolite V with platinum dioxide in methanol at 20° and 700 mm Hg, showed a maximum at 279 μm in its ultraviolet spectrum (characteristic of p-alkylphenols) and a band at 1775 cm⁻¹ in its infrared spectrum (characteristic of saturated γ-lactones). Finally, metabolic Product V was prepared by synthesis starting from 1-acetoxy-3-(4-acetoxyphenyl)-2-propenoate (Compound VII, R=AcO--) as shown in Scheme 1.

Structure III. Compound III

Metabolite V, C₁₁H₁₄O₂N, was found to contain an α,β-unsaturated γ-lactone (ultraviolet spectrum with absorption maximum at 215 μm; infrared spectrum with absorption bands at 1790, 1750, and 1640 cm⁻¹; positive reaction in the Legal and Bush tests) and a monosubstituted aromatic ring (bands at 730 and 700 cm⁻¹ in its infrared spectrum). Structure V was confirmed by comparison of the metabolite product with a synthetic sample of 3-benzyl-4-hydroxy-2-butenoic acid lactone, obtained, as shown in Scheme 1, by some modifications of the method of Plattner (8).

Structure IV. Compound IV

Metabolite V, C₁₁H₁₄O₂N, was found to contain an α,β-unsaturated γ-lactone (ultraviolet spectrum with absorption maximum at 208 μm; infrared spectrum with absorption bands at 1810, 1720, and 1640 cm⁻¹; positive reaction in the Legal and Bush tests) and a hydroxyl group on the benzene ring (infrared spectrum with absorption band at 3300 cm⁻¹; positive reaction in the Millon test). The hydroxyl group appears to be placed in the para position of the benzene ring because the ultraviolet spectrum of Metabolite V showed a second absorption maximum at 279 μm (ε 2410); p-cresol has λ_max 280 μm (11), whereas m- and o-cresol have λ_max at 274 μm (11). Further support for Structure V derives from the nuclear magnetic resonance spectrum of this compound showing absorptions at 3.65 δ (2H, —CH=—C=CH—), 4.75 δ (2H, —O—CH=—C=CH—), 5.90 δ (vinyl protons), 6.65 δ (phenolic proton), and 7.15 δ (3 aromatic protons) but no signal corresponding to a methyl group at 1.5 to 2.0 δ. The dihydroderivative of Metabolite V, obtained by hydrogenation of Metabolite V with platinum dioxide in methanol at 20° and 750 mm Hg, showed a maximum at 279 μm in its ultraviolet spectrum (characteristic of p-alkylphenols) and a band at 1775 cm⁻¹ in its infrared spectrum (characteristic of saturated γ-lactones). Finally, metabolic Product V was prepared by synthesis starting from 1-acetoxy-3-(4'-acetoxyphenyl)-2-propenoate (Compound VII, R=AcO--)

DISCUSSION

The foregoing spectroscopic and synthetic evidences prove the proposed structural formulae for the four metabolites (II, III, IV, and V) isolated from urine of rat and rabbit treated orally with 3-methyl-4-phenyl-3-butenamide (Compound I). These metabolic products are absent from the urine of rat and rabbit before treatment with the drug. Since in the urine of man receiving Compound I the same four metabolites were identified, this fact indicates that the metabolic pathway of Compound I is probably rather general. Whereas Compounds II and III are formed by the usual biological hydroxylations (12), two mechanisms seem likely for the metabolic formation of Lactone IV, and at this time they appear experimentally indistinguishable. In the former, according to Barton, Beckwith, and Goussen (13), the carbonyl function would have a direct role in inserting oxygen at the methyl group in γ-position for subsequent lactonization. However in the latter, Lactone IV could be formed by an independent hydroxylation of the methyl group followed by lactonization. Work is in progress in order to distinguish between the two mechanisms.

Lactone V is, at least in part, produced by hydroxylation of Lactone IV; in fact, this metabolic transformation has been experimentally confirmed. Nevertheless, at present, another mechanism, involving the metabolic transformation of Compound V from Compound III, cannot be excluded.

REFERENCES

Metabolism of 3-Methyl-4-phenyl-3-butenamide
L. Canonica, P. Manitto, U. Valcavi and N. Zonta Bolego


Access the most updated version of this article at http://www.jbc.org/content/243/7/1645

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/243/7/1645.full.html#ref-list-1