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

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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-butenoic acid lactone (IV), and 3-(4′-hydroxybenzyl)-4-hydroxy-2-butenoic 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) (1) is a hypocholesterolemic and hypolipemic agent (2–4) 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 examining the chromatogram in ultraviolet light or spraying it with 3,5-dinitrobenzoic acid according to Bush (6). Legal and Millon tests were carried out according to Feigl (7).

Urine was collected daily and was kept at 0° and pH 7 after the addition of chloroform. The pooled urine was extracted with chloroform at pH 7.8 and the chloroform extract was washed with water, then dried over anhydrous sodium sulfate, and finally evaporated to dryness. Column chromatography of the residue was carried out with Florisil[1] as adsorbent and elution with the following sequence of eluents: benzene, benzene-ethyl ether (1:1); benzene-ethyl ether (1:5); ethyl ether, ethyl ether-ethyl acetate (2:1); ethyl ether-ethyl acetate (1:1); ethyl ether-ethyl acetate (1:2); ethyl acetate.

Metabolism of 3-Methyl-4-phenyl-3-butenamide (Compound I) in Rat—Twenty-two male rats weighing 240 to 260 g received orally 0.5 g of Compound I per kg daily for 18 days; the pooled urine (3.6 liters) gave, when worked up as described above, 5.7 g of residue. This material was chromatographed on 200 g of Florisil. The fraction eluted with benzene-ethyl ether, 1:1 (600 ml), afforded 0.67 g of 3-benzyl-4-hydroxy-2-butenoic acid lactone (Compound IV), $b_{0.1}$ 149°; positive reaction in the Legal and Bush tests; λ_{max}, 215 mμ (ε 10,000); ν_{max}, 3030, 1790, 1750, 1640, 1600, 1490, 1450, 1250, 1120, 730, and 700 cm^{-1}.

$C_{11}H_{10}O_2$ (174.19)

Calculated: C 75.84, H 5.78
Found: C 75.72, H 5.82

The fraction eluted with benzene-ethyl ether, 1:5 (600 ml), gave 0.5 g of 3-(4′-hydroxybenzyl)-4-hydroxy-2-butenoic acid lactone (Compound V), m.p. 125–126°; positive reaction in the Legal, Millon, and Bush tests; λ_{max}, 208 mμ (ε 24,000), 279 mμ (ε 2410); ν_{max}, 3300, 1810, 1720, 1640, 1620, 1600, 1510, and 1260 cm^{-1}; in nuclear magnetic resonance spectrum bands at 3.65 (singlet, 2H, —CH_2—C=CH—), 4.75 (singlet, 2H, —O—CH_2—C=CH—), 5.90 (broad singlet, 1H, C=CH—CO_2—), 6.65 (phenolic OH), 7.15 (broad singlet, 4H, aromatic protons).

$C_{11}H_{10}O_3$ (190.19)

Calculated: C 69.49, H 5.30
Found: C 69.28, H 5.20

The fraction eluted with ethyl ether-ethyl acetate, 2:1 (600 ml), gave 0.25 g of 3-methyl-4-phenyl-3-butenamide (Compound I), m.p. 132–133°, λ_{max}, 204 mμ (ε 21,300), 245 mμ (ε 16,500); ν_{max}, 3400, 3200, 1660, 1180, 750, and 705 cm^{-1}; in nuclear magnetic resonance spectrum bands at 2.07 (doublet, J = 1.5 cps, 3H, CH_3—C=CH—), 3.35 (singlet, 2H, —CH_2—$CONH_2$), 6.60 (broad singlet, 1H, —C=CH—), 7.32 (broad singlet, 5H, aromatic protons), 8.0 (very broad singlet, 2H, —$CONH_2$).

The fraction eluted with ethyl ether-ethyl acetate, 1:1 (600 ml), yielded 0.5 g of 2-hydroxy-3-methyl-4-phenyl-3-butenamide

[1] Floridin Company, 60 to 100 mesh.

(Compound II), m.p. 162–163°; no reaction with Legal and Millon tests; λ_{max}, 205 mμ (ε 21,600), 245 mμ (ε 17,600); ν_{max}, 3450, 3350, 3200, 1670, 1190, 1020, 760, and 705 cm^{-1}; in nuclear magnetic resonance spectrum bands at 2.20 (doublet, J = 1.5 cps, 3H, CH_3—C=CH—), 5.12 (singlet, 1H, —CH(OH)—$CONH_2$), 6.98 (broad singlet, 1H, —C=CH—), 7.35 (broad singlet, 5H, aromatic protons), 8.20 (very broad singlet, 2H, —$CONH_2$).

$C_{11}H_{13}O_2N$ (191.22)

Calculated: C 69.08, H 6.85, N 7.32
Found: C 69.20, H 7.05, N 7.41

The fraction eluted with ethyl ether-ethyl acetate, 1:2 (700 ml), gave 0.6 g of 3-methyl-4-(4′-hydroxyphenyl)-3-butenamide (Compound III), m.p. 161–162°; no reaction with Legal and Bush tests; positive reaction with Millon test; λ_{max}, 213 mμ (ε 11,300), 257 mμ (ε 19,800); ν_{max}, 3300, 1670, 1610, 1520, 1230, 1180, 1100, 885, 860, and 820 cm^{-1}; in nuclear magnetic resonance spectrum bands at 2.15 (doublet, J = 1, 5 cps, 3H, CH_3—C=CH—), 3.38 (singlet, 2H, —CH_2—$CONH_2$), 6.65 (broad singlet, 1H, —C=CH—), 7.30 (broad singlet, 4H, aromatic protons), 8.10 (very broad singlet, 2H, —$CONH_2$).

$C_{11}H_{13}O_2N$ (191.22)

Calculated: C 69.08, H 6.85, N 7.32
Found: C 68.89, H 7.05, N 7.08

The same four metabolic 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 3-Benzyl-4-hydroxy-2-butenoic acid lactone (Compound IV)—1-Hydroxy-3-phenyl-2-propanone acetate (Compound VII, R=H) (26.45 g) (8) 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 (159 ml), then refluxed again for 15 min, cooled, and finally decanted in a mixture of ethyl ether (560 ml) and hydrochloric acid 4% in water (560 ml). The ethereal 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° and at 20 mm Hg of pressure, a residue was obtained which was extracted with ethyl ether. This ethereal solution was washed with water and $NaHCO_3$, 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° 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-(4′-Hydroxybenzyl)-4-hydroxy-2-butenoic Acid Lactone (Compound V)—4-Acetoxyphenylacetic acid chloride (Compound VI, R=AcO—) (10.4 g), prepared according to Heilbron and Cook (9) and Heilborn, Cook, and Elvidge (10), was treated with diazomethane (obtained from 30 g of *N*-nitroso-*N*-methylurea) in methylene chloride (400 ml). After stirring the mixture for 2 hours at 20°, the solvent was evaporated at room temperature and the residue was heated with acetic acid (100 ml) at 100° for 30 min. After evaporation of the acetic acid at 50° and dilution with water, the mixture was extracted with ethyl ether. The ethereal solution was washed with water, then with $NaHCO_3$ 5% in water, and with water again and finally evaporated to dryness. Crystallization of the residue from ethyl ether-*n*-hexane gave 1-acetoxy-3-(4′-acetoxyphenyl)-2-propanone (Compound VII, R=AcO—) (8.2 g), m.p. 59–60°.

$C_{13}H_{14}O_5$ (250.24)

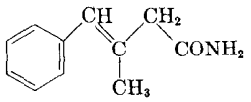
Calculated: C 62.40, H 5.63
Found: C 62.66, H 5.67

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 V isolated from the urine as a metabolite of Compound I.

Synthesis of 2-Hydroxy-3-methyl-4-phenyl-3-butenamide (Compound II)—3-Methyl-4-phenyl-3-butenamide (Compound I) (20 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

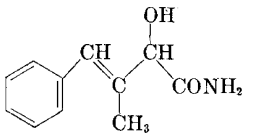
From the urine of rat and rabbit treated with 3-methyl-4-phenyl-3-butenamide (Compound I) four metabolic products have been isolated.



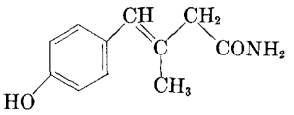
STRUCTURE I. Compound I

Metabolite II, $C_{11}H_{13}O_2N$, 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 mμ), 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_3—C=CH—), but, instead of the band at 3.35 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.

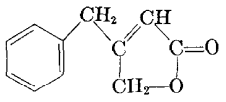


Structure II. Compound II

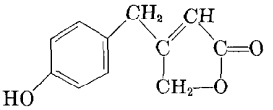


Structure III. Compound III

Metabolite III, $C_{11}H_{13}O_2N$, 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 mμ), and a hydroxyl group in the *para* position of the benzene ring (positive reaction in the Millon test; infrared spectrum with absorption band at 860 cm^{-1}). Further support for Structure III was obtained from the nuclear magnetic resonance spectrum of this metabolite, showing, similar to Compound I, bands at 2.15 (3H, CH_3—C=CH—) and at 3.38 δ (2H, —C=C—CH_2—$CONH_2$), but a band at 7.30 δ corresponding to 4 aromatic protons only. Finally, the ultraviolet spectrum of Compound III is in agreement with the structure proposed: in fact, it shows an absorption maximum at 257 mμ (ε 19,800), which can be compared with those of *p*-hydroxy-(1-propenyl)benzene (λ_{max} 259 mμ, ε 18,600) (11), *m*-hydroxy-(1-propenyl)benzene (λ_{max} 250 mμ, ε 6,000) (11), and *o*-hydroxy-(1-propenyl)benzene (λ_{max} 250 mμ, ε 10,000) (11). Metabolite IV, $C_{11}H_{10}O_2$, was found to contain an α,β-unsaturated γ-lactone (ultraviolet spectrum with absorption maximum at 215 mμ; 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 IV was confirmed by comparison of the metabolic 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).



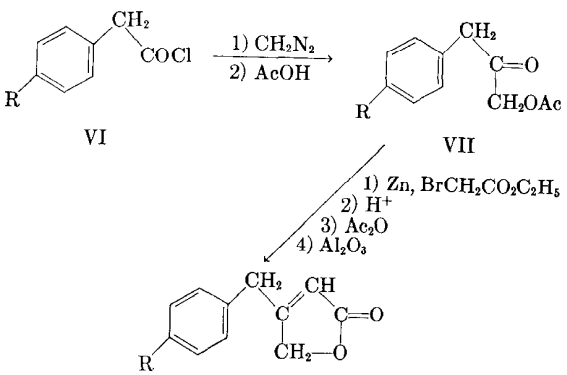
Structure IV. Compound IV



Structure V. Compound V

Metabolite V, $C_{11}H_{10}O_3$, was found to contain an α,β-unsaturated γ-lactone (ultraviolet spectrum with absorption maximum at 208 mμ; 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 mμ (ε 2410): *p*-cresol has λ_{max} 280 mμ (11), whereas *m*- and *o*-cresol have λ_{max} at 274 mμ (11). Further support for Structure V derives from the nuclear magnetic resonance spectrum of this compound showing signals at 3.65 δ (2H, —CH_2—C=CH—), 4.75 δ (2H, —O—CH_2—C=CH—), 5.90 δ (vinylic proton), 6.65 δ (phenolic proton), and 7.15 δ (4 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 mμ 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-propanone (Compound VII, R=AcO—) as shown in Scheme 1.

Scheme 1



VI VII

IV: R = H
V: R = OH

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 Goosen (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

1. Canonica, L., Bonati, A., Gaudenzi, L., and Motta, G., *Il Farmaco, Ed. Sci.*, **14**, 112 (1959).
2. Canonica, L., Santi, R., and Scarselli, V., in *Proceedings of the International Symposium on Drugs Affecting Lipid Metabolism*, Milan, 1960, p. 328.

3. Sanguinetti, F., and Zannoni, M. L., *Boll. Soc. Ital. Biol. Sper.*, **38,** 982 (1962).
4. Sanguinetti, F., and Zannoni, M. L., *Minerva Med.*, **54,** 2985 (1963).
5. Rossi, C. S., *Boll. Soc. Ital. Biol. Sper.*, **36,** 1914 (1960).
6. Bush, I. E., and Taylor, D. A. H., *Biochem. J.*, **52,** 643 (1952).
7. Feigl, F., *Spot tests in organic analysis*, Ed. VI, Amsterdam, 1960, pp. 196, 236.
8. Plattner, P., and Heusser, H., *Helv. Chim. Acta*, **28,** 1044 (1945).
9. Heilbron, I. M., and Cook, A. H., *Chem. Abstr.*, **42,** 617 (1948).
10. Heilbron, I. M., Cook, A. H., and Elvidge, I. A., *Chem. Abstr.*, **42,** 8215 (1948).
11. Kamlet, M. J., *Organic electronic spectral data*, Interscience Publishers, New York, 1960.
12. Tecwyn Williams, R., *Detoxication mechanisms*, Ed. II, Chapman and Hall, London, 1959, p. 151.
13. Barton, D. H. R., Beckwith, A. L. J., and Goosen, A., *J. Chem. Soc.*, 181 (1965).