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METABOLISM OF A NEW SYNTHETIC PROGESTAGEN, ORG 2969, BY HUMAN LIVER *IN VITRO*

LASSE VIINIKKA
Department of Clinical Chemistry, University of Oulu, SF-90220 Oulu 22, Finland

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SUMMARY

The *in vitro* metabolism of a new synthetic progestagen, Org 2969 (13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol), was studied in human liver homogenate. The metabolites formed were purified by t.l.c. and identified using capillary g.l.c. and g.l.c.-mass spectrometry of the O-methyl oxime trimethylsilyl ether and trimethylsilyl ether derivatives. Four metabolites, 3α- and 3β-hydroxy-Org 2969, 3-oxo-Org 2969 and 3α-hydroxy-5α-H-Org 2969 were identified. The identification of a fifth metabolite, present in low concentrations, 3-oxo-5α-H-Org 2969 was tentative. On the basis of the results, a scheme for the metabolism of Org 2969 is suggested: Org 2969 is first hydroxylated to 3α/3β-hydroxy-Org 2969, followed by oxidation to 3-oxo-Org 2969, which is further reduced to 3α-hydroxy-5α-H-Org 2969 via 3-oxo-5α-H-Org 2969. Since Org 2969 is only bound relatively weakly by myometrial progesterone receptor, while the affinity of 3-oxo-Org 2969 is high, it has been assumed that at least partly the high biological activity of Org 2969 *in vivo* is mediated via biotransformation to its 3-oxo derivative. The results of the present investigation indicate the possibility of such a biotransformation in humans.

INTRODUCTION

Org 2969 (13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol) (Fig. 1) is a new orally active progestagen synthesized in the laboratories of Organon Int., Oss, The Netherlands. The compound has proved to be a very potent progestational agent in animal experiments [1]. In clinical experiments [2–4] its ovulation inhibiting activity has also been observed to be very high, which together with the lack of harmful side effects has made it a very promising compound for clinical use.

The aim of the present investigation was to study the metabolism of Org 2969 using human liver homogenate. Special interest was paid to the possible formation of 3-oxo-Org 2969, because another orally active 3-deoxyprogestagen, lynestrenol, is known to be metabolized to its 3-oxo derivative [5, 6].

It is assumed that the high biological activity of Org 2969 is at least partly mediated via bioactivation to metabolites, such as 3-oxo-Org 2969, because the affinity of Org 2969 to myometrial progesterone receptor is comparatively low, only 16% and 18% of that of progesterone in the human and rabbit, respectively, whereas the affinity of 3-oxo-Org 2969 is very high, i.e., 150% and 280%, respectively [2].

The following codes and trivial names were used: Org 2969 = 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol; 3-oxo-Org 2969 = 13-ethyl-17-hydroxy-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3-one; 3α-hydroxy-Org 2969 = 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yne-3α,17-diol; 3β-hydroxy-Org 2969 = 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yne-3β,17-diol; 3-oxo-5α-H-Org 2969 = 13-ethyl-17-hydroxy-11-methylene-18,19-dinor-5α,17α-pregn-20-yn-3-one; 3α-hydroxy-5α-H-Org 2969 = 13-ethyl-11-methylene-18,19-dinor-5α,17α-pregn-20-yne-3α,17-diol; lynestrenol = 19-nor-17α-pregn-4-en-20-yn-17-ol; stigmasterol = (24R)-24-ethyl-5,22-cholestadien-3β-ol.

EXPERIMENTAL

Solvents. All solvents were of reagent grade, and were redistilled before use, apart from ethanol and propylene glycol which were used as supplied.

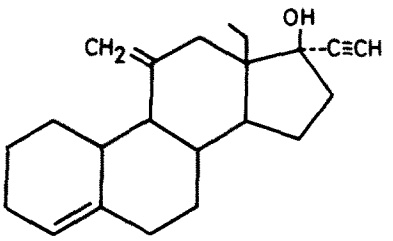
Reference steroids. Org 2969, [16-^{3}H]-Org 2969 and all the presumed metabolites used as standards were donated by Dr. E. de Jager, Organon Scientific Development Group, Organon, Oss, The Netherlands.

Thin layer plates (Kieselgel 60 F 254) were from Merck AG, Darmstadt, GFR, and were washed by eluting them with methanol before use.

Gas liquid chromatography (g.l.c.) was performed with a 25 m long, 1% OV-101 capillary column (Prolab Oy, SF-02700 Kauniainen, Finland) with a programmable Carlo Erba gas chromatograph, model 2403 T.

Gas chromatography-mass spectrometry (GC-MS). A Jeol JMS D 100 mass spectrometer connected to a Carlo Erba gas chromatograph 2403 T and coupled to a Jeol Mass Data System was used.

A liver sample was obtained from a 43 year old woman, who was killed by accident and brought to the Department of Forensic Medicine, University of Oulu. A 10 g sample was obtained at the autopsy (12 h after death). It was washed with ice cold 0.25 M sucrose in 0.067 M phosphate buffer, pH 7.4, cut into



ORG 2969

Fig. 1. The structure of Org 2969.

pieces and homogenized with an Ultra-Turrax-type homogenizer for 30 seconds in 4 vol. of ice cold buffer. The post-mitochondrial 10,000 *g* supernatant obtained by centrifugation at 4°C for 20 min was used as a source of enzymes.

Incubation was carried out at 37°C in an atmosphere of 95% O_2 and 5% CO_2 in the phosphate buffer mentioned above. NADP (1.1 mmol/l), glucose-6-phosphate (2.2 mmol/l), ATP (0.11 mmol/l) and 10 units of glucose-6-phosphate dehydrogenase were added to 40 ml of incubation fluid. The steroid substrate, 3 mg of [16-^{3}H]-Org 2969 (4.5 μCi) was added in 1 ml of ethanol–propylene glycol (40:60, V/V) to the incubation medium. The incubation was terminated by extracting aliquots from the incubation medium twice with 2 vol. of ethyl acetate 3, 10 and 30 min after the start of the incubation. In every aliquot less than 5% of the radioactivity remained in the aqueous phase.

Purification and identification of the metabolites. After extraction the organic phases were combined and evaporated to dryness *in vacuo*. The dry residues were redissolved in 0.5 ml aliquots of ethyl acetate, and were transferred to thin layer plates. The plates were developed once using chloroform–acetone (90:10, V/V) as the solvent system. The plates were dried and the radioactive spots were localized with a Philips thin layer scanner PW 4007. The radioactive spots were eluted with ethanol. The steroids were then converted to trimethylsilyl ether (TMS) or O-methyl oxime trimethylsilyl ether derivatives (MO-TMS) [7, 8]. The identification of the metabolites by g.l.c. and GC-MS was based on identical retention times and mass spectra with those of reference compounds. The metabolites were quantitated using stigmasterol as internal standard, assuming equimolar amounts of the compounds giving the same peak area.

RESULTS

The disappearance of the parent compound, Org 2969, from the incubation medium is shown in Fig. 2. After 3, 10 and 30 min, the respective percentages of the compound still unchanged were 93%, 77% and 57% respectively. Eight to nine metabolites were found. The concentrations of five of these during the course of the incubation are shown in Fig. 2. The identification of the metabolites was based on the following findings.

Compound I

Compound **I** did not form an O-methyl oxime derivative. The peaks observed at high mass region in the mass spectrum of compound I-TMS (Fig. 3), suggested a molecular peak (M) at mass 470. The peaks at mass 455 (M-15) and 441 (M-29), corresponding

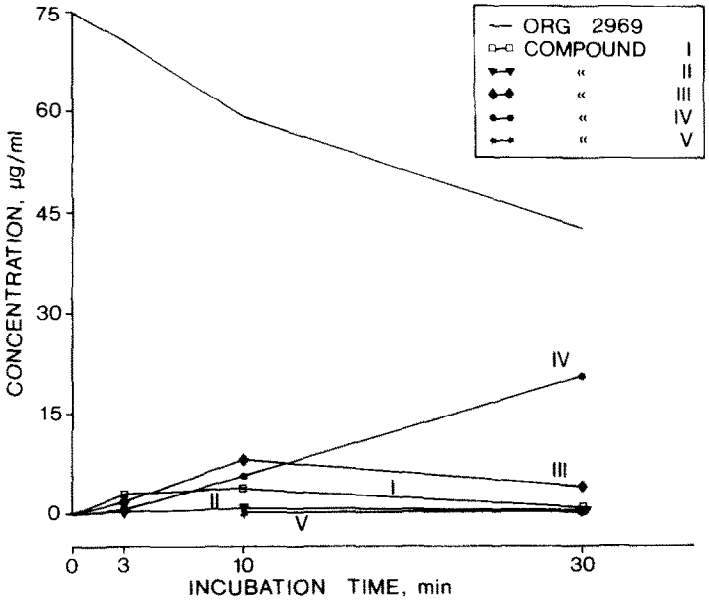


Fig. 2. The concentrations of Org 2969 and its main metabolites during the incubation with human liver. Values are expressed as μg/ml of the incubation medium. Compound **I** = 3α-hydroxy-Org 2969, Compound **II** = 3β-hydroxy-Org 2969, compound **III** = 3-oxo-Org 2969, compound **IV** = 3α-hydroxy-5α-H-Org 2969, compound **V** = 3-oxo-5α-H-Org 2969 (only tentatively identified).

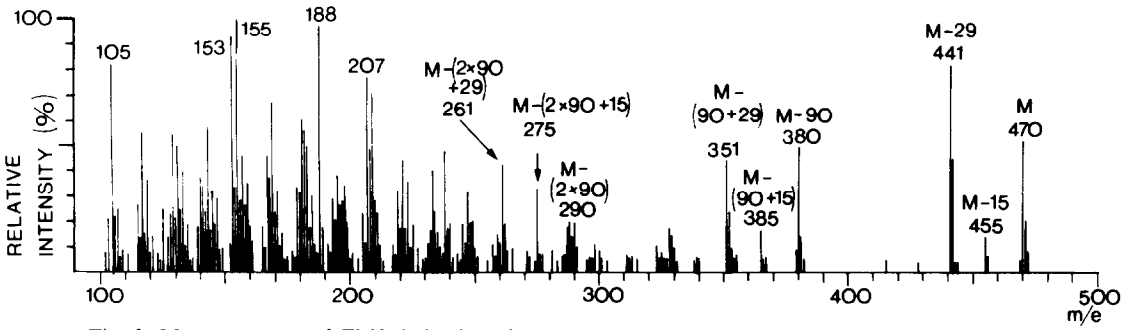


Fig. 3. Mass spectrum of TMS-derivative of compound **I**, which was identified as 3α-hydroxy-Org 2969.

to the elimination of methyl- and 13-ethyl-radicals from the molecular ion upon electron impact, are characteristic for TMS ether derivatives of Org 2969 compounds, respectively. Loss of trimethylsilanol molecules (mass 90) up to a maximum of two from the ions M, (M-15) and (M-29) indicated the presence of two trimethylsilylated hydroxyl groups in the molecule. Comparison of the mass spectrum of compound **I**-TMS with the mass spectra of the trimethylsilylated reference compounds, showed that the mass spectrum of **I**-TMS was identical with the spectrum of trimethylsilylated 3α-hydroxy-Org 2969. The retention time of the TMS-derivative of compound **I** was also identical with the corresponding derivative of this reference compound. Consequently, compound **I** was identified as 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yne-3α,17-diol.

Compound **II**

The fragmentation of compound **II** during mass spectrometry of its TMS derivative was very similar to that of compound **I** suggesting that compound **I** and compound **II** are epimeric compounds. The retention time was identical with that of the TMS-derivative of the reference compound, 3β-hydroxy-Org 2969, and therefore compound **II** was identified as 3β-hydroxy-Org 2969 (13-ethyl-11-methylene- 18,19-dinor-17α-pregn-4-en-20-yne-3β,17-diol).

Compound **III**

The molecular peak in the mass spectrum of the MO-TMS derivative of compound **III** was observed at mass 425 (Fig. 4). Further evidence for the presence of one $CH_3ON<$ and one TMSO-group was supported by the fragment peaks at mass 394 (M-OCH_3); 335 (M-90) and 304 (M-90-31). The retention time and mass spectrum were identical with those of the corresponding derivative of 3-oxo-Org 2969, resulting in the identification of compound **III** as 13-ethyl-17-hydroxy-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3-one.

Compound **IV**

Compound **IV** did not form an O-methyl oxime derivative. The molecular peak of the TMS-derivative was observed at mass 472, while the fragmentation pattern indicated a structure with two trimethylsilylated hydroxyl groups (Fig. 5). The retention time and spectrum were identical with those of the TMS derivative of 3α-hydroxy-5α-H-Org 2969, and therefore compound **IV** was identified as 13-ethyl-11-methylene-18,19-dinor-5α,17α-pregn-20-yne-3α,17-diol.

Compound **V**

Because of the low concentration of compound **V**, it was only possible to obtain a mass spectrum of bad quality. The molecular peak of the MO-TMS derivative was at *m/e* 427, which suggests the presence of a saturated steroid nucleus with one oxo and one hydroxyl group in the underivatized sample. The retention time was identical with that of the corresponding derivative of 3-keto-5α-H-Org 2969, and its structure is tentatively identified as 13-ethyl-17-hyd-

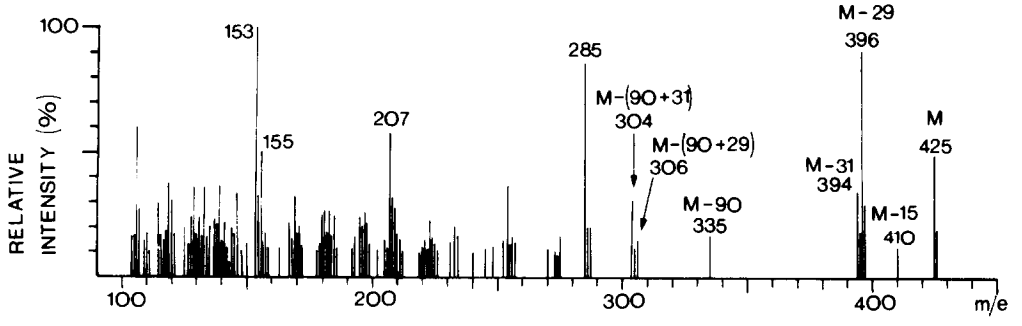


Fig. 4. Mass spectrum of MO-TMS-derivative of compound **III**, which was identified as 3-oxo-Org 2969.

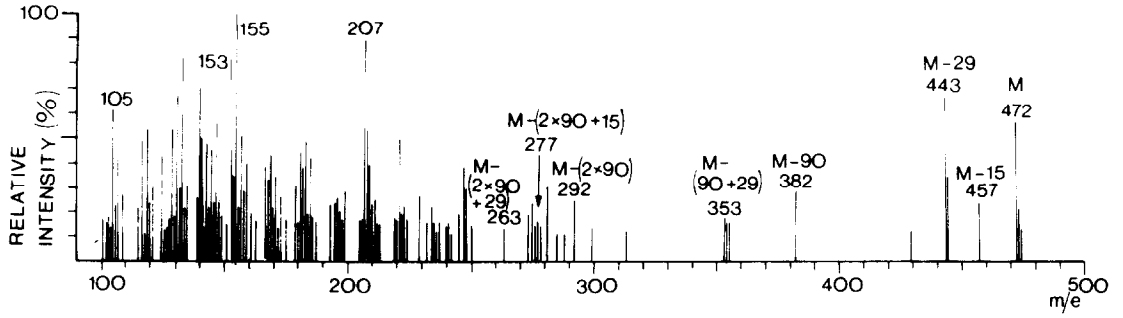


Fig. 5. Mass spectrum of TMS-derivative of compound **IV**, which was identified as 3α-hydroxy-5α-H-Org 2969.

roxy-11-methylene-18,19-dinor-5α,17α-pregn-20-yn-3-one.

In addition to compounds **I-V**, 3–4 compounds with clearly higher polarities than compounds I–V, or of the standards available, appeared in the incubation fluid 30 min after the start of the incubation. It is assumed that these compounds were polyhydroxylated, unconjugated metabolites of Org 2969, but because of the lack of the reference compounds, no definite identification could be carried out.

DISCUSSION

The results of the present investigation suggest that the metabolic pathways of Org 2969 are as presented in Fig. 6. Since a 3-deoxy-structure does not exist as a function of endogenous steroids in man the direct introduction of an oxo function at carbon three of the steroid nucleus is difficult to assume. It is assumed that the first reaction is the formation of a hydroxyl group (Fig. 6, reactions A_1 and A_2). This kind of reaction, the mechanism of which is known in principle, is common in the metabolism of various compounds by liver microsomes (for a review, see ref. 9). The hydroxylation is then followed by dehydrogenation to 3-oxo-Org 2969 (Fig. 6, reactions B_1 and B_2). The dehydrogenation of both 3α-hydroxy-4-ene and 3β-hydroxy-4-ene steroids to the corresponding 3-oxo-derivative by the liver has been described earlier [10]. The suggested pathway from Org 2969 to 3-oxo-Org 2969 is very similar to that proposed for lynestrenol by Mazaheri *et al.*[6].

The reduction of 3α-hydroxy-Org 2969 to 3α-hydroxy-5α-H-Org 2969 (Fig. 6, reaction C) is also a possible pathway. The latter compound could also be formed by reduction of 3-oxo-Org 2969 (Fig. 6, reactions D and E). No evidence of the practical importance of this reaction sequence can be derived from the results of the present experiment. No definite conclusions can be drawn about the reversibility of the

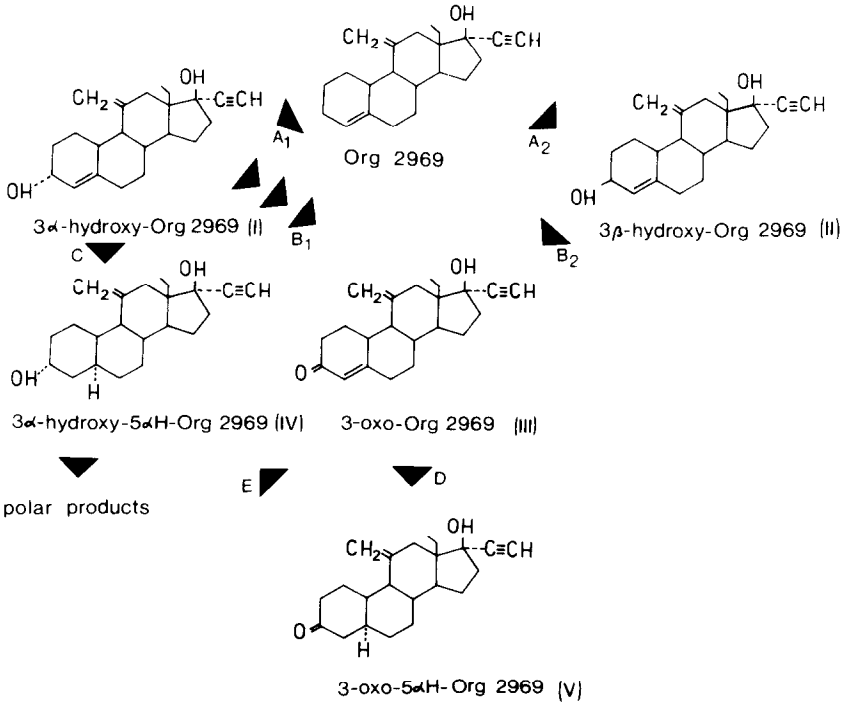


Fig. 6. The suggested metabolic pathways of Org 2969 in human liver *in vitro*.

reactions described, because no attempts were made to clarify the equilibrium kinetics of these reactions in this study. Theoretically it is evident that these reactions proceed mainly in one direction, because there is a tendency to change apolar products into more polar ones to facilitate excretion.

Since Org 2969 is bound only relatively weakly by the myometrial progesterone receptor, while the affinity of 3-oxo-Org 2969 is high [2], it has been assumed that the high biological activity of Org 2969 *in vivo* is at least partly mediated by the biotransformation to its 3-oxo-derivative. The results of the present investigation indicate the presence of such a biotransformation in human liver. Therefore, liver metabolism might be one of the origins for 3-oxo-derivative of Org 2969 present in human serum after administration of the parent compound [11]. It is not expected that the other metabolites identified contribute to the biological activity of Org 2969 to any great extent, since the changes in 3-oxo-4-ene structure decrease the affinity of a steroid to the receptor considerably [12].

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REFERENCES

1. De Visser J., de Jager E., de Jongh H. P., van der Vies J. and Zeelen F.: Pharmacological profile of a new orally active progestational steroid: Org 2969. *Acta endocr., Copenh. Suppl.* **199** (1975) 405.
2. Viinikka L., Ylikorkala O., Nummi S., Virkkunen P., Ranta T., Alapiessa U. and Vihko R.: Biological effects of a new and potent progestagen. A clinical study. *Acta endocr., Copenh.* **83** (1976) 429–438.
3. Skouby S.: The influence on the pituitary-ovarian function, cervical mucus and vaginal cytology of a new progestational compound. *Contraception* **14** (1976) 529–539.
4. Viinikka L., Hirvonen E., Ylikorkala O., Nummi S., Virkkunen P., Ranta T., Alapiessa U. and Vihko R.: Ovulation inhibition by a new low dose progestagen. *Contraception* **16** (1977) 51–58.
5. Murata S.: Studies about the metabolism of estrane-progestins. *Folia Endocr. Jap.* **43** (1967) 1083–1096.
6. Mazaheri A., Fotherby K. and Chapman J.: Metabolism of lynestrenol to norethisterone by liver homogenate. *J. Endocr.* **47** (1970) 251–252.
7. Vihko R.: Gas chromatographic mass spectrometric studies on solvolyzable steroids in human peripheral plasma. *Acta endocr., Copenh. Suppl.* **109** (1966) 1–67.
8. Fales H. M. and Luukkainen T.: O-methyloximes as carbonyl derivatives in gas chromatography, mass spectrometry and nuclear magnetic resonance. *Analyt. Chem.* **37** (1965) 955–957.
9. White A., Handler P. and Smith E.: *Principles of Biochemistry*, 5th Edn. McGraw-Hill, Kogakusha Ltd., Tokyo (1973) pp. 403–405.
10. Ungar F., Gut M. and Dorfman R. I.: Metabolism of Δ^4-androstene-3β,17β-diol and Δ^4-androstene-3α,17β-diol *in vitro*. *J. biol. Chem.* **224** (1957) 191–200.
11. Viinikka L.: Radioimmunoassay of a new progestagen, Org 2969, and its metabolite *J. steroid Biochem.* **9** (1978) 979–982.
12. Kontula K., Jänne O., Vihko R., de Jager E., de Visser J. and Zeelen F.: Progesterone binding proteins: *In vitro* binding and biological activity of different steroidal ligands. *Acta endocr., Copenh.* **78** (1975) 574–592.