Microbial transformation of cycloartenol and 24-methylene cycloartanol

4 AUTHORS, INCLUDING:

Shoei-Sheng Lee
National Taiwan University

196 PUBLICATIONS **2,241** CITATIONS

MICROBIAL TRANSFORMATION OF CYCLOARTENOL AND 24-METHYLENE CYCLOARTANOL

K. C. Wang,* Liang-Hwa Young, Yu Wang,† and Shoei-Sheng Lee
School of Pharmacy, College of Medicine, and† NSC X-ray Center, Department of Chemistry,
National Taiwan University, Taipei 10018, Taiwan, R.O.C.

Summary: Cycloartenol (I) and 24-methylenecycloartanol (II) are the major triterpene alcohols present in the rice bran oil. Upon exposure of I, II and lanosterol to the growing culture of *Mycobacterium sp.* (NRRL B-3805), androsta-4,8(14)-diene-3, 17-dione (III) was isolated and characterized as the major metabolite in 34%, 35% and 30% yield, respectively.

It is well known that a wide range of microorganisms are able to utilize cholesterol and phytosterols as the sole carbon source for growth and propagation.[1,2] It is also well documented that in the steroid degrading microorganisms, the ring structure and side chain are metabolized by different enzyme systems simultaneously and independently.[3] In recent years, methods for selective cleavage of the sterol side chains by microorganisms, such as (i) modification of sterol structure,[4] (ii) addition of enzyme inhibitors,[5-7] and (iii) employment of mutants,[8-9] were developed to give C_{19} and/ or other useful steroid intermediates. The brilliant work of Sih and coworkers [10] led us gain an insight into the pathways and mechanisms involved in the microbial degradation of sterol side chains.

Cycloartenol (I) and 24-methylenecycloartanol (II) are the major triterpene alcohols present in the rice bran oil.[11] In both compounds, the C-19 carbon is incorporated into a cyclopropyl ring with C-9 and C-10 carbons and there are three methyl groups located at 4-,4- and 14α- positions of steroid nucleus. Upon exposure of compound I and II to the growing culture of *Mycobacterium sp* (NRRL B-3805),[8,12] compound III was isolated from the incubation mixture as the major metabolite in 34% and 35% yield, respectively.
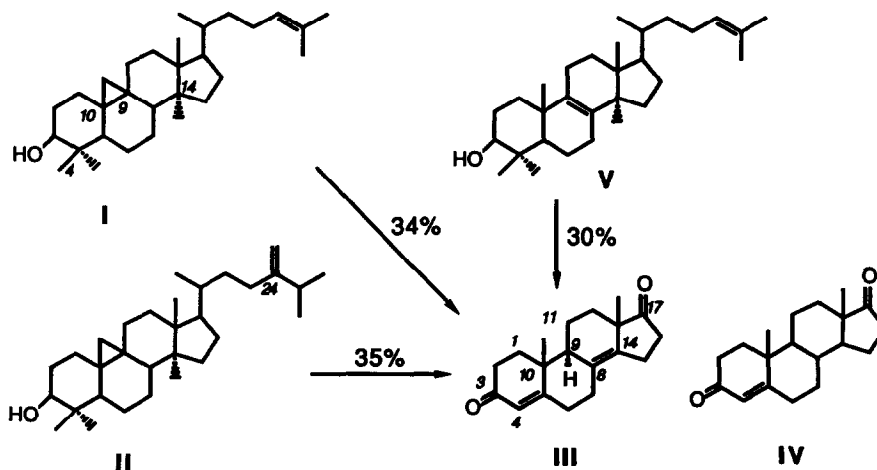
Compound III,[13] mp. 141-142° C from diethyl ether, shows in its high resolution mass spectrum a molecular ion at m/z 284.2765, corresponding to a formula of $C_{19}H_{24}O_2$ (calcd. 284.1776). Its uv absorption maximum at 233.0 nm and ir absorption at 1669 cm^{-1} indicate an α,β-unsaturated carbonyl chromophore.[14] Another ir absorption at 1742 cm^{-1} suggests the presence of a five-membered non-conjugated carbonyl.[14] These two structural moieties are supported by NMR spectra, of which the signal of an olefinic proton at δ 5.75 (br. s, 4-H), and the carbon signals at δ 198.7 (3-C), 169.6 (5-C) and 123.4 (4-C) constitute an α,β-unsaturated carbonyl system, and the signal at δ 220.6 represents the remaining non-conjugated carbonyl. In addition, ^{1}H-NMR indicates it contains two methyls (δ 1.11 and 1.10). These spectral features suggest that compound III is an analogue of androst-4-ene-3,17-dione (IV) which was the major metabolite from the incubation of cholesterol with *Mycobacterium sp* (NRRL B-3805).[8] While comparing their formula, III contains two less hydrogens than IV, indicating that III has one more double bond equivalent . The ^{13}C-NMR spectrum of III accounts for this difference by showing an extra fully substituted double bond (δ 136.7 and 127.8) and lacking two methines' signals in comparison with IV. From structural analysis, the fully substituted double bond in III can be only disposed between either C-8 and C-9 or between C-8 and C-14.

Several structures are possible to fit the above data. A hetero-long range COSY spectrum optimized for J_{CH} = 10 Hz indicates that the methyl protons at δ 1.11 is coupled to 17-C (δ 220.6), 14-C (an olefinic carbon, δ 136.7), 13-C (a quaternary carbon, δ 47.0), and 12-C (a methylene carbon, δ 28.7), whereas the other methyl protons at δ 1.10 is coupled to 5-C (δ 169.6), 1-C (a methylene carbon, δ 34.2) and 10-C (a quaternary carbon, δ 39.9). These data suggest that the fully substituted double bond is located between C-8 and C-14, and the two methyls (δ 1.10 and 1.11) are linked to C-10 and C-13, respectively. Thus, except the stereochemistry of C-9 remaining to be clarified, the skeleton of III is determined.

Chemical models indicate that if 9-H (δ 2.09, br. t, J = 8.4 Hz, designated from homo-COSY) is α-oriented, 11β-H (δ 1.57, ddt, J = 14.0, 14.0, 10.3, 3.6 Hz, assigned from nOe studies and homo-COSY), a pseudoaxial proton, will be spatially close to 18-H's and 19-H's. Thus, nOe experiments upon irradiation either at 18-H's or 19-H's would enhance the intensity of 11β-H. On the other hand if 9-H is β-oriented, the above nOe effects will not be observed. The actual nOe experiments show positive enhancements of 11β-H upon irradiation at 18-H's (δ 1.11) or 19-H's (δ 1.10). Consequently, 9-H is α-oriented and compound III is androsta-4,8(14)-diene-3,17-dione.
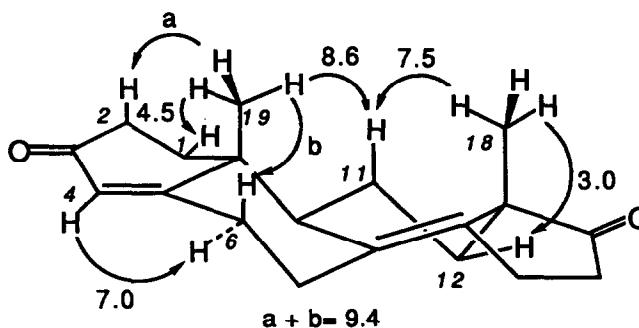
NOE's (%) of compound III (CDCl₃, Bruker 300 AM)

This structure assignment is confirmed by single crystal X-ray crystallography.[15]

Isolation of III from the incubation mixtures showed that the following changes had taken place: (i) the cyclopropyl ring was opened to form the C-19 methyl; (ii) three methyl groups located on the steroid nucleus were demethylated; (iii) two double bonds, Δ^4 and $\Delta^{8(14)}$, were introduced, and (iv) the side chains were cleaved to give 17-ketone. These transformations except (iv) are first observed with *Mycobacterium sp.* (NRRL B-3805).

Lanosterol (V), an intermediate in the cholesterol biosynthesis with three methyl groups identical to those of I and II on the nucleus and has a $\Delta^{8(9)}$ double bond but without the cyclopropyl ring, appears to be an interesting substrate for transformation by this microorganism. Incubation of V with *Mycobacterium sp.* (NRRL B-3805) also gave compound III as the major metabolite isolable of 30% yield.

Currently, we are in the process of isolation and characterization of minor metabolites from the incubation mixtures for further information concerning this transformation.

References

1. L. L. Smith in Biotechnology Vol. 6a. Biotransformation, **1984**, p.31, edited by H. J. Rehm and G. Reed, Verlag Chemie.
2. K. Kieslich, *J. Basic Microbiol.*, **1985**, *25*, 461.
3. C. J. Sih and H. W. Whitlock, *Ann. Rev. Biochem.* **1968**, *37*, 661.
4. (a) C. J. Sih and K. C. Wang, *J. Amer. Chem. Soc.*, **1965**, *87*, 1387; (b) C. J. Sih, S. S. Lee, Y. Y. Tsong, K. C. Wang and F. N. Chang, *ibid*, **1965**, *87*, 2765.
5. G. Wix, K. G. Buki, E. Tomorkeny and G. Ambrus, *Steroids*, **1968**, *11*, 401.
6. (a) K. Arima, M. Nagasawa, N. Bae and G. Tamura, *Agr. Biol. Chem.*, **1969**, *33*, 1636; (b) M. Nagasawa, M. Bae, G. Tamura and K. Arima, *ibid*, **1969**, *33*, 1644.
7. Van der W. F. Waard, J. Doodewarrd, de J. Flines and van der S. Weele, Abh. Deut. *Akad. Wis. Berlin Kl. Med.*,**1969**, *2*, 101.
8. (a) W. J. Marsheck, S. Krachy and R. D. Muir, *Appl. Microbiol*, **1972**, *23*, 72; also (b) *US Patent* **1972**, 3,684,657 and **1973**, 3,759,791.
9. M.G. Wovcha, F. J. Antosz, J. C. Knight, L.A. Komined and T. R. Pyke, *Biochem. Biophys. Acta*, **1978**, *531*, 308.
10. (a) C. J. Sih, K. C. Wang and H.H. Tai, *J. Amer. Chem. Soc.*, **1967**, *89*, 1956 ; (b) C. J. Sih, H. H. Tai and Y. Y. Tsong, *ibid.*, **1967**, *89*, 1957; (c) Y. Fujimoto, C. S. Chen, A. G. Gopalan and C. J. Sih, *ibid*, **1982**, *104*, 4718 ; (d) Y. Fujimoto, C. S. Chen, A. G. Gopakan and C. J. Sih, *ibid*, **1982**, *104*, 4720.
11. T. Itoh, T. Tamura and T. Matsumoto, *J. Amer. Oil. Chem. Soc.*, **1973**, *50*, 300.
12. The microorganism was maintained on a Bacteria Agar (Dextrose 1%, yeast extract 1%, Nutrient Broth 0.8% and Agar 3.5%) at 26ºC for 11 days then transfered and grown in Nutrient broth- Dextrose medium (Nutrient broth 1.6% and Dextrose 4%) at 26- 28º C on a rotary shaker (250 rpm, 1-in stroke). Transformation was carried out in 2-L Erlenmyer flasks containing 400 ml of medium. The substrate dissolved in DMF was added to the growing microorganism and the incubation was continued for 5 days.
13. The physical data of III were obtained from the following instruments: $[\alpha]_D$: JASCO DIP-181 Digital Polarimeter; m.p.: Buchi 510 Melting Apparatus; ir: Jasco A-100 Infrared Spectrophotometer; uv: Hitachi 150-20 Double Beam Spectrophotometer; mass: Finnigan Mat 4500 series GC/MS and Jeol JMS-HX110 Mass Spectrometer; NMR: Bruker AC-80 and AM-300. Some physical data of III: $[\alpha]_D^{20}$ +316º (c 0.25, EtOH);

ir: λ_{max}(KBr, cm^{-1}) 2961, 1742, 1669, 1610, 1447, 1370; uv : λ_{max} (MeOH, log ε) 233.0 nm (4.07); EIMS: m/z (%) 285 ([M+1]$^{+\cdot}$, 68.2), 284 (M$^{+\cdot}$, 100.0), 227 (24.3), 212 (81.0), 209 (61.0), 199 (22.1); ^{1}H-NMR assignment: 1.80 (1α), 1.95 (1β), 2.35 (2α and 2β), 5.75 (4), 2.39 (6α and 6β), 1.91 (7α), 2.71 (7β), 2.09 (9), 1.73 (11α), 1.57 (11β), 1.21 (12α), 1.78 (12β), 2.18 (15α), 2.58 (15β), 2.78 (16α), 2.55 (16β), 1.11 (18), 1.10 (19). ^{13}C-NMR assignment: 34.2 (C-1), 33.8 (C-2), 198.7 (C-3), 123.4 (C-4), 169.6 (C-5), 32.3 (C-6), 28.8 (C-7), 127.8 (C-8), 47.6 (C-9), 39.9 (C-10), 18.4 (C-11), 28.7 (C-12), 47.0 (C-13), 136.7 (C-14), 35.8 (C-15), 22.9 (C-16), 220.6 (C-17), 21.8 (C-18) and 18.2 (C-19). These assignments were made by nOe and 2D NMR techniques and will be described somewhere else in more details.

14. K. Nakanishi, Infrared Absorption Spectroscopy, **1976**, p.42, 1st ed, Holden-Day, San Francisco.

15. Crystal data of compound III: orthorhombic P 2,2,2; a= 8.994 (3), b= 10.483 (2), c= 16.680 (4) Å, Z= 4, Intensity data were collected on a CAD-4 diffractometer with θ /2θ scan mode, using monochromated MoKα radiation. Data were measured up to 2θ of 50°. A total of 1607 reflections were collected. Among them, 637 were considered to be observed (> 1.5 σ (I)). Final agreement indices are R(F)= 0.064, WR (F)= 0.056, S= 2.7, based on anisotropic refinement of all non-hydrogen atoms. The list of atomic co-ordinates and molecular dimensions have been deposited with Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW.