the adjacent sp hybridized carbon. Ring expansions from four- to five-membered rings are very common, and a number of rationalizations for the selectivity of the ring expansions have been advanced.[18] In our examples, it appears that the non-vinyl or non-aryl carbon, a in **11**, selectively migrates in every case.[14] While the ability of the migrating group to stabilize positive charge probably plays some role in governing the selectivity of the reaction, we can explain the outcome of the ring expansion by postulating a reaction path that proceeds through the best stabilized cationic intermediate. Then, formation of the final product is concluded in a stereospecific fashion by trans addition across the alkyne bond as depicted in **12**.

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About the Mechanism of Sterol Biosynthesis

Alain Krief,*[†] Jean-Roch Schauder,[†] Eric Guittet,[‡] Catherine Herve du Penhoat,[‡] and Jean-Yves Lallemand[‡]

Department of Chemistry, Facultês Universitaires Notre-Dame de la Paix 61, rue de Bruxelles, B-5000 Namur, Belgium Laboratoire de RMN, Institut de Chimie des Substances Naturelles, F-91190 Gif-sur-Yvette, France

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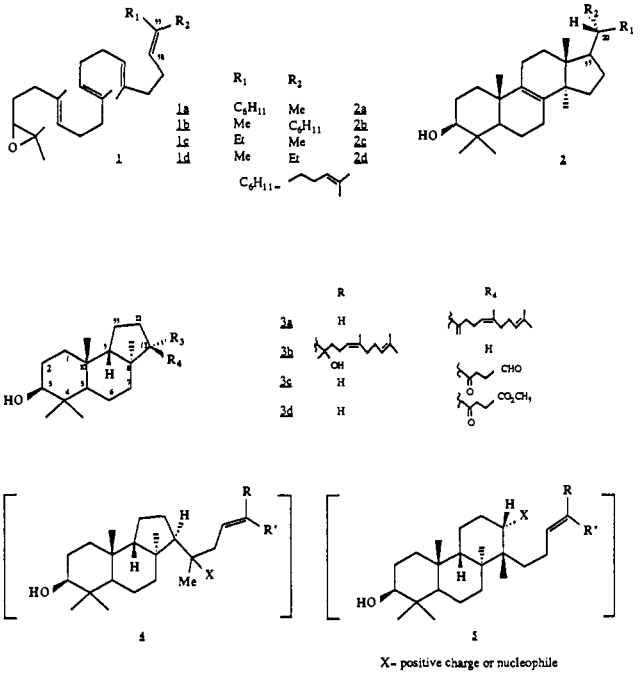
The transformation of 2,3-oxido-(*all trans*)-squalene **1a** to lanosterol **2a** possessing not less than seven asymmetric centers has been the subject of intensive work since the first hypothesis of Woodward and Bloch and the brilliant theoretical models proposed[1] independently by the Zurich School and by Stork.

Although several aspects of these hypotheses have been supported by careful experiments with radiolabeled 2,3-oxidosqualene or with analogous lacking one or several methyl groups,[1] the problems related to the C-20 carbon atom of lonosterol have not yet been clarified. It is well established however that the cyclization process ends at this position and also that a 120° rotation of the alkyl chain around the C-17–C-20 bond must precede the series of migrations in order to achieve the 20 (*R*) stereochemistry found in lanosterol.[1]

In a previous study we found[2] that the truncated 2,3-oxidosqualene analogues **1c** and **1d** possessing $\Delta^{18\text{-}19}$ double bonds with the natural *E* and the unnatural *Z* stereochemistry produce the tetranorlanosterols **2c** and **2d**, respectively, having the natural 20 (*R*) and the unnatural 20 (*S*) stereochemistry. As a continuation of this work it became particularly important to study the behavior of the 2,3-oxidosqualene **1b** with the complete hydrocarbon framework but with the unnatural *Z* stereochemistry at $\Delta^{18\text{-}19}$.

This compound possessing a tritium radiolabel at C-3 has been prepared from the commercially available pure *all-trans*-farnesol (**3a**) as the basic subunit by using the well-established Biellman method[3] for the construction of the complete skeleton possessing the correct oxidation level at C-2 and at C-3 and a *Z* $\Delta^{18\text{-}19}$ carbon–carbon double bond. We have adopted as the key step an unusual strategy purposely implying a nonregioselective oxidation of the *trans,trans*-farnesol in order to achieve *in one step* the differentiation between the two different subunits required.[4]



Anaerobic incubation of the racemic labeled oxide **1b** (250 μg, 4.14×10^{-6} dpm) at 20 °C with a solution (6 mL) of oxidosqualene sterol cyclase[5] affords a mixture of compounds containing 92% of the initial radioactivity, which exhibits on thin-layer radiochromatography on SiO_2 (eluted with benzene/ethyl acetate, 9/1) four major radioactive spots: (i) a fraction A which corresponds to the unchanged labeled oxide (59%, R_f 0.84), (ii) a fraction B (6%, R_f 0.50), (iii) a fraction C (16%) whose R_f (0.43) is close to that of lanosterol (R_f 0.44), and (iv) a more polar fraction D (19%, R_f 0.29).

On silver nitrate impregnated SiO_2 TLC (eluted with benzene/ethyl acetate, 8:2), fraction B (R_f 0.34), fraction C (R_f 0.52), and fraction D (R_f 0.20) are all more polar than lanosterol or its iso 20 (*S*) stereoisomer[6] (R_f 0.70). The complete absence of the latter two compounds in our biosynthetic mixture was further unambiguously confirmed by |GC|[2] and HPLC experiments which include co-injection with authentic samples.[6] It is therefore clear that this biosynthesis takes a different course from that of 2,3-oxido-(*all trans*)-squalene (**1a**) or from that of 2,3-oxidotetranorsqualene (**1d**) possessing the $\Delta^{18\text{-}19}$ *Z* stereochemistry.

Fractions B, C, and D contain several exogeneous products, but each fraction presents a major radiolabeled derivative. These compounds have been separated by HPLC (column Varian RP 18, 50 × 1 cm, eluted with acetonitrile, flow rate 5 mL/min, UV detector 200 nm, *D* (Rt 20 mn), *B* (Rt 24 mn) and *C* (Rt 33 mn)). After several large scale experiments and purification of the crude mixtures on PLC then on HPLC according to the previously

[†] Facultês Universitaires Notre-Dame de la Paix.

[‡] Institut de Chimie des Substances Naturelles.

(1) For a review article, see: Mulheirn, L. J.; Ramm, P. J. *Chem. Soc. Rev.* **1972**, 259.

(2) Herin, M.; Delbar, P.; Remion, J.; Sandra, P.; Krief, A. *Tetrahedron Lett.* **1979**, 1073.

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(4) The details of this synthesis will be reported later.

(5) (a) Hogebomm, S. H. *Methods in Enzymology*; Colowich, S. P., Kaplan, N. O., Eds.; Academic Press: 1955; Vol. 1, p 16. (b) A phosphate-buffered microsomal solution (70 mL) was prepared in the standard manner[5a] from minced hog liver (700 g) without any further purification. We are indebt to S. Wattiaux (Medical Faculty, Namur) for her help for the preparation of the microsomal solution. (c) **1b** remains unchanged if the enzymic solution is boiled for 0.3 h prior incubation.

(6) Schauder, J. R.; Krief, A. *Tetrahedron Lett.* **1982**, *23*, 4389.

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described protocol, we obtained milligram quantities (3, 8, 14 mg, respectively) of the analytically pure compounds *B*, *C*, and *D*.

We have not yet determined the structure of the minor component *B*, but we have assigned the tricyclic 6.6.5 structures **3a** and **3b**, respectively, to compounds *C* and *D* on the basis of their spectroscopical data and reactivity. We have been able to get quite good crystals of **3b** but were unable to get suitable X-ray data even with a synchrotron apparatus.

Both **3a** and **3b** exhibit closely related mass spectra.[7] Their ^1H and ^{13}C NMR spectra clearly suggest[8] that the cyclization process has only involved the epoxide ring, the Δ^{6-7}, the Δ^{10-11}, and the Δ^{14-15} carbon–carbon double bonds, leaving unaffected the Δ^{18-19} *Z* and the Δ^{22-23} carbon–carbon double bonds originally present on the oxidosqualene **1b**. They furthermore imply[8,9a] the presence of an extra terminal carbon–carbon double bond on **3a**.

Their IR spectra exhibit an absorption at 3580 cm^{-1} suggesting the presence of hydroxyl moieties on each compound. This is corroborated by their polar nature (see above the description of their behavior on SiO_2 which is close to that of lanosterol) as well as by their ^1H and ^{13}C NMR spectra.[8,9b]

3a and **3b** are monoacetylated after reaction with an excess of acetic anhydride in pyridine (20 °C, 17 h; acetate of **3a**: 87% yield, R_f 0.88; acetate of **3b**: 92% yield, R_f 0.42 on SiO_2 TLC, benzene/ethyl acetate, 95:5). Under the same conditions lanosteryl acetate has an R_f of 0.91, and even under more drastic conditions (excess reagent and longer reaction time) compound **3b** cannot be diacetylated. This behavior supports the assignment of the hindered tertiary alcohol (C-14 hydroxyl group) on **3b**.

The discrimination in favor of 6.6.5 arrangements come from the NMR studies which are disclosed on the accompanying paper[8] as well as from the typical fragmentations observed on the mass spectra of the related compounds **3c** and **3d** available by chemical degradation[10] of the side chain of **3a**.

The stereochemistry of all the ring junctions of both **3a** and **3b** has been deduced from the interpretation of their 2D NMR which is presented in the accompanying paper.[8] Both **3a** and **3b** exhibit trans/syn/trans A/B/C ring junctions. However, although it has not yet been unambiguously secured, their stereochemistry at C-13 seems to be different.[8]

The β orientation of the side chain in **3a** leads us to assume that the cyclizing enzyme(s) operated normally[2,3] on **1b** insofar as possible and cyclized it from the chair–boat–chair interrupted conformation. The major biocyclized component **3b** if it possesses the α-oriented side chain would arise from an "abnormal" chair–boat–boat folding. At this point the stereochemistry at C-13 of **3a** and **3b** must be firmly established. We are therefore planning to prepare related crystalline derivatives suitable for X-ray analysis.

For the first time (i) tricyclic compounds are formed by enzymatic cyclization of an oxidosqualene analogue possessing the complete set of carbon–carbon double bonds, and (ii) the length of the hydrocarbon chain attached to the *Z* Δ^{18-19} carbon–carbon double bond of 2,3-oxidosqualene analogues is shown to have a dramatic influence on the nature of the enzymic products. This effect has never been observed[1,2,11] in the *all-trans* series of **1**.

This result let us to presume that in the enzyme, 2,3-oxidosqualenes **1** are first cyclized to tricyclic 6.6.5 intermediates **4**, which then rearrange to 6.6.6 intermediates **5** precursors of the tetracyclic pattern of steroids when an extra stabilization arising, for example, from the Δ^{18-19} carbon–carbon double bond, is available.[12] This stabilization is missing when the Δ^{18-19} carbon–carbon double bond is missing,[13-15] and in fact a compound with a 6.6.5 arrangement of the A/B/C rings is isolated suggesting that in **1b** the side chain is, for steric reasons, unable to attain the required conformation which would allow this extra stabilization. With this respect, compounds **3a** and **3b** obtained during this study would be ideal for testing this hypothesis. They will be transformed into their analogues possessing an *E* rather than a *Z* trisubstituted carbon–carbon double bond in the hope that they would lead to tetracyclic derivatives on further reaction with oxidosqualene sterol cyclase.[16]

(7) Mass spectra of **3a**: 426, 408, 365, 283, 271, 257, 247, 229, 213, 203, 189, 187, 175, 161, 147, 121, 119, 107, 95, 81, 69, 55. For **3b**: 426, 408, 393, 365, 339, 283, 271, 257, 247, 229, 204, 203, 189, 175, 161, 147, 135, 121, 119, 107, 95, 81, 69, 55. We acknowledge Dr. P. Sandra (University of Gent) for these measurements.

(8) Guittet, E.; Herve du Penhoat, C.; Lallemand, J.-Y.; Schauder, J. R.; Krief, A., following paper in this issue.

(9) (a) ^1H NMR: two singlets at 4.85 and 4.62 ppm, attributed to the hydrogen linked to the terminal carbon–carbon double bond; ^{13}C NMR: two signals at 149.20 and 109.06 ppm attributed to the two sp^2 olefinic carbon atoms. (b) ^{13}C NMR of **3a**: 79.25 (C-3); of **3b**: 79.40 (C-3) and 76.45 ppm (C-14).

(10) By ozonolysis of **3a** (O_3/CH_2Cl_2 then Me_2S), then oxidation of resulting keto aldehyde **3c** with Jones' reagent (2 equiv, acetone, 0 °C, 0.3 h), and then esterification (CH_2N_2/ether, 20 °C, 14% overall yield of **3d**).

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(15) (a) The action of the cyclase on 15′-nor-18,19-dihydro-2,3-oxidosqualene leads[15b] however to a tricyclic compound possessing a 6.6.6 arrangement. (b) van Tamelen, E. E. *J. Am. Chem. Soc.* **1982**, *104*, 6480 and references cited.

(16) We thank I.R.S.I.A. Belgium for a fellowship to J. R. Schauder and the FNRS (Belgium) for financial support.

Application of Homonuclear Chemical Shift Correlation NMR to the Study of the Cyclization Products of Unnatural Δ^{18-19}-(*Z*)-Epoxysqualene

Eric Guittet,[†] Catherine Herve du Penhoat,[†] Jean-Yves Lallemand,[†] Jean-Roch Schauder,[‡] and Alain Krief*[‡]

Laboratoire de RMN, Institut de Chimie des Substances Naturelles F-91190 Gif-sur-Yvette, France
Facultés Universitaires Notre-Dame de la Paix Laboratoire de Chimie Organique 61, rue de Bruxelles, B-5000 Namur, Belgium

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Interpretation of the results of the biological cyclization of 2,3-oxidosqualene analogues possessing the Δ^{18-19}-*Z* stereochemistry[1] required the structural elucidation of the products. Surprisingly enough, although NMR studies of macromolecules of biological interest is widespread, the complete structural elucidation of some comparatively small molecules such as saturated functionalized steroid-type systems remains a real challenge. This communication describes the structural determination of three tricyclic derivatives **1**, **2**, and **3** based on a combination of force-field calculations and 2D NMR, an approach easily extended to related compounds.

From ^1H[2] and ^{13}C[3] 1D NMR and mass spectra it appeared that compound **1** contained a tertiary and a quaternary hydroxyl group, two trisubstituted double bonds, and consequently three cycles.

A multiple quantum-filtered COSY experiment[4] revealed several distinct proton filiations (Figure 1). The intact fragment corresponding to carbons 16–24, 29, and 30 of starting epoxy-

[†] Institut de Chimie des Substances Naturelles.

[‡] Facultés Universitaires Notre-Dame de la Paix.

(1) Krief, A.; Schauder, J. R.; Guittet, E.; Herve du Penhoat, C.; Lallemand, J. Y., preceding paper in this issue.

(2) $CDCl_3$: five methyl groups at 0.878, 1.036, 1.068, 1.157, and 1.225 ppm; allylic methyl groups at 1.706 (3 H) and 1.782 (6 H) ppm; one proton α to a hydroxyl group at 3.310 ppm (d × d, 11.5 and 5.2 Hz); two ethylenic protons at 5.21 ppm (d × d, 8.0 and 8.0 Hz).

(3) C_6D_6: 26 sp3 carbons; two carbons bearing a heteroatom at 75.8 (d) and 79.2 (s) ppm.

(4) Piantini, U.; Soerensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800–6801.

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