The Use of ^{1}H NMR for Yield Determination in the Regioselective Epoxidation of Squalene

Hans A.J. Aerts, Bert F.E. Sels, and Pierre A. Jacobs*

Centre for Surface Chemistry and Catalysis, Katholieke Universiteit Leuven, 3001 Heverlee, Belgium

ABSTRACT: ^{1}H NMR can be used to determine the epoxide yield rapidly in the oxidation of squalene. Moreover, unequivocal distinction can be made between internal and terminal epoxide bonds. To underline the power of this technique, different stoichiometric and catalytic epoxidation procedures were carried out using squalene as substrate. They were characterized in terms of substrate conversion and regioselectivity of the epoxide fraction.

Paper no. J10990 in *JAOCS 82*, 409–413 (June 2005).

KEY WORDS: 2,3-Epoxides, ^{1}H NMR, regioselective epoxidation, squalene.

Besides the double bond epoxidation of fatty compounds (1), we became interested in the epoxidation of squalene and more specifically in the search for new catalytic regioselective epoxidation procedures for this compound. Squalene is a key intermediate in the biosynthesis of steroids such as lanosterol and cholesterol (2). The first step in this sequence consists of the formation of the terminal epoxide (2,3-oxidosqualene) by the squalene epoxidase enzyme (3). Although the synthetic production of this epoxide is unusually demanding owing to the similarity of the double bonds in the molecule, some authors have succeeded in the high-yield production of the terminal epoxide using *N*-bromosuccinimide (NBS) in a glyme/water solvent mixture followed by a base-catalyzed dehydrobromination (4,5).

A great deal of research has been done on the driving force behind this unique regioselectivity. Although the first studies ascribed the selective formation of the terminal epoxide to a specific coiling of the squalene molecule, it is also accepted that the polarity of the oxidant plays a role in the product selectivity. Thus, the formation of both internal and terminal epoxides is associated with the ability of the oxidant to penetrate the coiled molecule. Charged or highly polarized oxidants such as NBS only affect the ends of the squalene molecule, yielding only the terminal epoxides. A third explanation for the highly selective NBS/glyme/water system (6) is based on the heat of formation of internal and terminal epoxides, which is reduced in the latter case by 5 kcal/mmol. During our research we encountered the problem of analysis. Because a facile analysis by GC is not possible, we screened the literature thoroughly. Surprisingly, only one procedure for the analysis of the epoxide isomers is reported (4). Although this method is widely applied, it is time-consuming and not very accurate. For example, the epoxide is first hydrolyzed to obtain the diol using aqueous perchloric acid. In a second step, the diol is quantitively converted into acetone and aldehydes. Finally, the terminal epoxide yield can be determined by measuring the amount of acetone present, whereas the internal epoxides are quantified based on the aldehydes. Nowadays, different types of NMR can be used to identify and characterize squalene and its epoxides. Although important work has been reported (7,8), no effort has been made to quantify the molar ratio of the internal vs. terminal epoxides when present in an isomeric mixture.

In this article, we report the determination of the internal and terminal epoxide yields in squalene oxidation using ^{1}H NMR. For illustrative purposes, a range of catalytic and stoichiometric epoxidation methods was tested. With the NMR method, we were able to determine the ratio of the internal vs. the terminal epoxide yield. Compared with the currently used method, ^{1}H NMR analysis is more accurate and less time-consuming.

EXPERIMENTAL PROCEDURES

Materials. Squalene (>98%) was purchased from Sigma; *m*-chloroperbenzoic acid (*m*CPBA; 70–75%), aqueous H_2O_2 (35%), $CDCl_3$ (1% tetramethylsilane), Oxone®, decane, hexadecyltrimethylammonium chloride, KOH, and Na_2SO_4 were bought at Acros. Tungstic acid, hexadecyltrimethylammonium bromide, tetraethoxysilane (TES), diglyme, diethylether, dodecyldimethylephidrinium bromide, *N*-benzyldimethylhexadecylammonium chloride, and *N*-benzyldimethyltetradecylammonium chloride were purchased from Fluka; H_3PO_4 (85%), CH_2Cl_2 (DCM), tetrabutylorthotitanate (TBOT) from Merck; tricaprylmethylammonium chloride were from Aldrich; ethanol, methanol, and $NaHCO_3$ were from BDH; and aqueous ammonia (25%) was from Riedel.

Synthesis of PW_4-PTC. This synthesis was carried out according to Venturello and D'Aloisio (9). A suspension of tungstic acid (10 mmol) in 7 mL of 35% aqueous H_2O_2 is stirred and heated to 60°C until a colorless solution is obtained. To this solution, filtered and cooled to room temperature, is added 2.5 mmol of 85% H_3PO_4. The whole is diluted

---

*To whom correspondence should be addressed at Centre for Surface Chemistry and Catalysis, Kasteelpark Arenberg 23, 3001 Heverlee, Belgium. E-mail: Pierre.Jacobs@agr.kuleuven.ac.be to 30 mL, and 5 mmol of a quaternary ammonium (PTC), e.g., tricaprylmethylammonium chloride in 40 mL of DCM is added dropwise while stirring. The stirring is continued for 15 min, after which the organic phase is separated, dried using Na_2SO_4, and filtered. The solvent is removed under reduced pressure at 40—50°C. Based on the type of quaternary ammonium compound used, a gel or solid is obtained.

Synthesis of Ti-MCM-41 (Si/Ti = 2.5) (10,11). Hexadecyltrimethylammonium bromide (6.8 mmol) is dissolved in distilled water (50 g). After adding aqueous ammonium (0.25 mmol) and ethanol (1.3 mmol), the mixture is stirred for 15 min. To this solution, 22 mmol of TES and 2 mmol of TBOT are added. After stirring for 2 h at 0°C, the precipitate is filtered, washed with water and methanol, and dried at 60°C overnight. Finally, the catalyst is calcined at 500°C for 5 h (0.5°C/min).

Epoxidation using mCPBA. Squalene (2 mmol) is dissolved in 10 mL $CHCl_3$, and 0.32 mmol of *m*CPBA is added. The reaction mixture is stirred at room temperature for about 20 min after which water is added to remove the 3-chlorobenzoic acid. The organic layer is separated, dried with Na_2SO_4, and evaporated under reduced pressure.

Epoxidation using PW_4-PTC (9). Squalene (2 mmol) is added to 5 mL of DCM. To this solution, 0.018 mmol of PW_4-PTC catalyst is added together with 0.32 mmol of 35% aqueous H_2O_2. After 2 h at 40°C, the organic phase is separated, dried using Na_2SO_4, and evaporated under reduced pressure at 40–50°C.

Epoxidation using dioxirane chemistry. The procedure of Sonnet *et al.* (12) is followed: To 2 mmol of squalene in acetone is added 26 mmol of $NaHCO_3$. Under vigorous stirring in the dark at ambient temperature, 5 mmol of Oxone® dissolved in 6 mL distilled H_2O is added dropwise in 1 h. Stirring is continued for 2 h after which water is added and the phases are separated.

Epoxidation using NBS (4). Squalene (2 mmol) and NBS (0.33 mmol) are dissolved in a mixture of H_2O/diglyme/DCM (0.2:4:1 mL/mL/mL). After stirring for 30 min at room temperature, the formed bromohydrin is washed and extracted into diethylether. The organic layer is separated, dried with Na_2SO_4, and then 10 mL KOH in methanol is added. After 1 h, the epoxide is extracted with diethylether, and the organic phase is separated and evaporated under reduced pressure at 40—50°C.

Epoxidation using Ti-MCM-41. Dried Ti-MCM-41 catalyst (0.2 g) is added to 2 mL tertiary butyl hydroperoxide in toluene (6 mmol) together with 5 mmol of squalene. After 24 h at 80°C, the organic layer is separated and evaporated under reduced pressure at 40°C.

Epoxidation using Payne chemistry (13). To a solution of 1.5 mmol of squalene in 6 mL methanol are added 16 mmol acetonitrile, 1.5 mmol of $KHCO_3$, and 9 mmol of aqueous 35% H_2O_2. After 25 h at 0°C, the formed epoxide is extracted with CH_2Cl_2 and the solvent evaporated under reduced pressure.

Analysis. Small amounts of substrate and product mixtures were dissolved in $CDCl_3$. ^1H, ^{13}C, and 135° distortionless enhancement by polarization transfer (DEPT) NMR were taken on a 300 MHz Bruker Avance NMR spectrometer with a magnetic field of 7.05 T. Eight scans were sufficient to obtain clear ^1H spectra, but an accumulation of 250 scans was necessary to get clear ^{13}C NMR spectra.

RESULTS AND DISCUSSION

Squalene epoxides (SO) are prepared by known stoichiometric and catalyzed reactions of squalene with different types of oxidants. They are all analyzed using ^1H NMR. Figure 1 shows reference spectra of squalene, SO with only terminal functions, and SO with internal and terminal oxirane functions. The assignments of the chemical shifts of important protons for squalene and their internal/terminal epoxides are given in Scheme 1. For confirmation, Figure 2 shows the ^{13}C NMR spectrum taken from a SO molecule containing both internal and terminal oxirane rings and one containing only terminal oxirane rings. Only two carbon signals, $-\mathbf{C}HO\mathbf{C}(CH_3)_2$, are observed when only terminal epoxides are present (58 and 63.9 ppm) whereas four signals are observed in this region (58, 60.5, 63.1, and 63.9 ppm) when terminal $-\mathbf{C}HO\mathbf{C}(CH_3)_2$ as well as internal epoxides $-\mathbf{C}HO\mathbf{C}(CH_3)-$ are present. Peaks in the 15 to 40 ppm range originate from the presence of $-\mathbf{C}H_3$ and $-\mathbf{C}H_2-$, whereas those around 130 ppm are due to the double bond carbons. The different carbons in the 58–64 ppm region were easily determined by 135° DEPT NMR; disappearance of the signals at 58 and 60.5 ppm pointed out the $-CHO\mathbf{C}(CH_3)_n-$, whereas the positive signals at 63.1 and 63.9 ppm originate from $-\mathbf{C}HOC(CH_3)_n-$ (n = 1 or 2).

It is clear that reaction yields can be calculated from ^1H NMR by evaluating the appearing and disappearing signals. Reaction conversion, expressed as conversion of double bonds, can be calculated by evaluating the disappearance of the signal at 5.1 ppm ($-C\mathbf{H}{=}C\mathbf{H}-$, *m*) and the new arising epoxide signal at 2.6 ppm ($-C\mathbf{H}OC\mathbf{H}-$, *m*). Equation 1 can be used to calculate reaction conversion:

$$X(\%)=\left[\frac{\dfrac{1}{N_{2.6,t}}*\dfrac{A_{2.6,t}}{A_{S,t}}}{\dfrac{1}{N_{2.6,t}}*\dfrac{A_{2.6,t}}{A_{S,t}}+\dfrac{1}{N_{5.1,t}}*\dfrac{A_{5.1,t}}{A_{S,t}}}\right]*100 \qquad [1]$$

in which $A_{2.6,t}$ and $A_{5.1,t}$ represent the intensities of the resonance signals at 2.6 and 5.1 ppm and $N_{2.6,t}$ and $N_{5.1,t}$ are the amount of protons for which the peak stands ($N_{2.6,t}$ and $N_{5.1,t}$ are both 2). $A_{S,t}$ is the intensity of one of the signals from squalene (e.g., 1.59 ppm) or SO to standardize the intensities.

As for regioselectivity at low reaction conversion, two types of epoxides can be formed, internal and terminal epoxides. We have noticed that these epoxides can be easily differentiated based on the chemical shift of the methyl groups in the ^1H NMR spectrum. If only terminal epoxides (2,3-epoxides) are formed, two evenly intense signals at 1.23 and 1.29 ppm ($-CHOC(C\mathbf{H}_3)_2$) can be observed in the spectrum; the internal epoxides give a single signal at 1.25 ppm

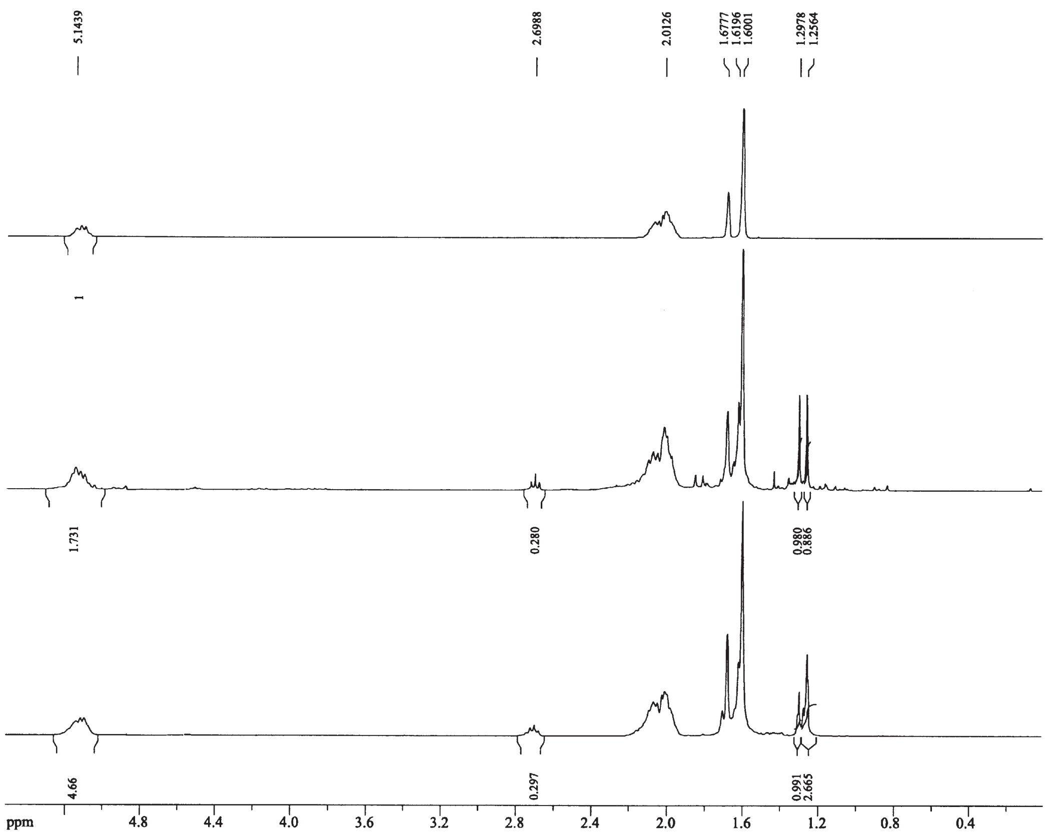


FIG. 1. ^{1}H NMR spectra of squalene (upper), squalene oxide (SO) containing only terminal oxirane rings (oxidation by N-bromosuccinimide) (middle), and SO containing internal and terminal epoxides (oxidation by Oxone®) (lower).

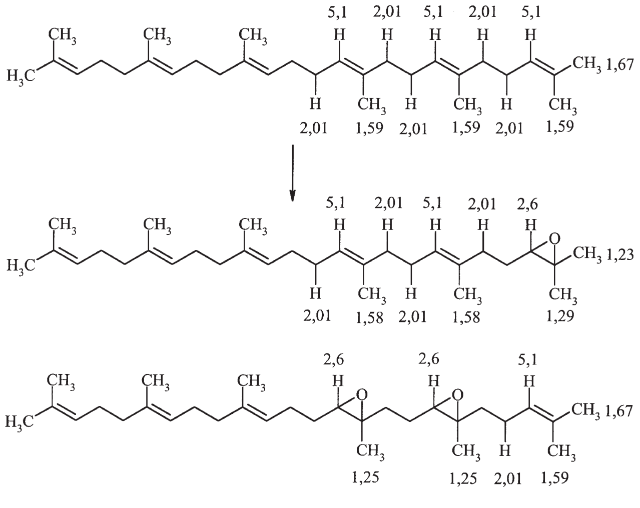
(–CHOC(C$\mathbf{H}_3$)–). From this, selectivity to the internal and terminal epoxide can easily be calculated using Equations 2 and 3:

$$S_{\text{internal}}(\%) = \left[\frac{\dfrac{1}{N_{\text{upfield},t}} * \dfrac{A_{\text{upfield},t}}{A_{S,t}} - \dfrac{1}{N_{\text{downfield},t}} * \dfrac{A_{\text{downfield},t}}{A_{S,t}}}{\dfrac{1}{N_{\text{upfield},t}} * \dfrac{A_{\text{upfield},t}}{A_{S,t}}} \right] * 100 \quad [2]$$

$$S_{\text{terminal}}(\%) = 100 = S_{\text{internal}} \quad [3]$$

in which $A_{\text{upfield},t}$ is the integration value of the entire cluster of peaks from approximately 1.26 to 1.20 ppm and $A_{\text{downfield},t}$ the intensity of the signal at 1.29 ppm. $N_{\text{upfield},t}$ and $N_{\text{downfield},t}$ present the amount of protons for which the peaks stand ($N_{\text{upfield},t}$ and $N_{\text{downfield},t}$ are both 3). $A_{S,\,t}$ is the intensity of one of the squalene (or SO) signals to standardize intensities. At a high degree of double bond conversion, more than one oxirane function appears in the molecule, giving rise to a small (~0.015 ppm) but equal downfield shift of the double singlet of the terminal methyl groups.

To underline the power of this analysis, oxidation procedures from the literature are repeated and compared with our results (Table 1). Because there are two internal (*I*) double bonds for each terminal double bond (*T*), some correction has to be made when calculating the ratio *T/I*; this is elucidated in Equation 4:



SCHEME 1

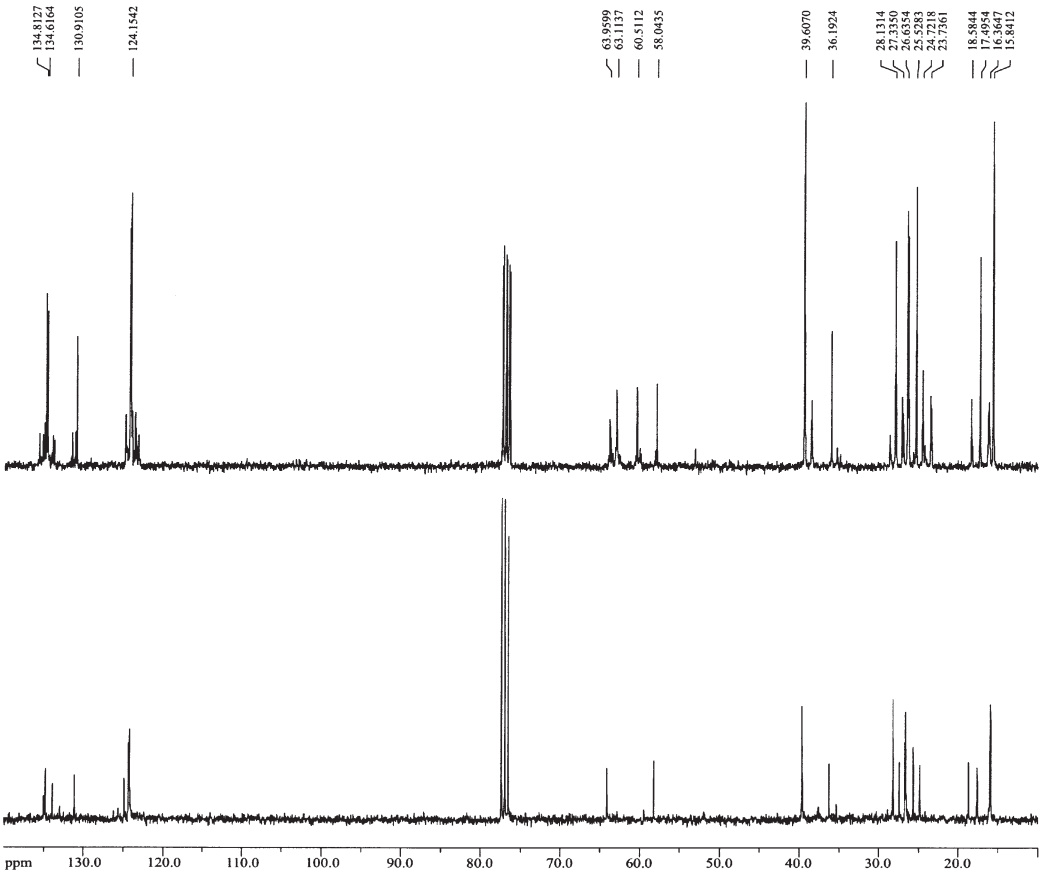


FIG. 2. ^{13}C NMR spectra of SO containing both internal and terminal epoxides (upper) and SO containing only terminal oxirane rings (lower). For abbreviation see Figure 1.

$$\frac{T}{I} = \text{ratio } T \textit{ vs } I = \frac{\%S_{\text{Ext}}/1}{\%S_{\text{Int}}/2} \qquad [4]$$

Our results (Table 1, entries 3 and 4) with NBS in water/diglyme/DCM and with peracid (Table 1, entry 1) confirm the literature results obtained by Van Tamelen and coworkers (4,5). If peracids are used, no regioselectivity is observed ($T/I = 1$). Thus, an equal formation of internal and terminal epoxides is obtained, whereas with NBS only terminal epoxides are found ($T/I = \infty$).

Because of the simplicity and the robustness of the analysis procedure, a whole range of common epoxidation methods were tested, which to the best of our knowledge have not been applied before in the regioselective epoxidation of squalene. The results are shown in Table 2. If the Venturello–Ishii PW_4-PTC catalysts (9) are used in a biphasic system in combination with aqueous hydrogen peroxide, a slight preference is found for the terminal epoxide (Table 2, entries 1–6). Within this group of catalysts, there is a clear dependence of the regioselectivity on the nature of the quaternary ammonium. For instance, trioctylmethylammonium leads to a *T/I* ratio of 3 whereas dodecyldimethylephidrinium gives a *T/I*

TABLE 1
Comparison of Oxidation Results Analyzed by ^1H NMR and Those Found in the Literature[a]

	Oxidant	^1H NMR results				Literature results			
		S_{internal} (%)	S_{terminal} (%)	Molar ratio *T/I*	*X* (%)	S_{internal} (%)	S_{terminal} (%)	Molar ratio *T/I*	*X* (%)
	Peracid								
1[b]	3-Chloroperbenzoic acid	33	66	1	16	42	58	1	N.A.
	NBS								
2[c]	NBS and base	100	0	∞	6	100	0	∞	N.A.
3	NBS and base	100	0	∞	13	100	0	∞	N.A.

[a] For analysis using ^1H NMR, a molar ratio of oxidant/double bond of 1:6 is used. H_2O/diglyme/DCM is used as solvent.
[b] In the literature, *trans,trans*-hexadeca-2,10-diene-2,6,15-trimethyl is used instead of squalene (4).
[c] Molar ratio oxidant/double bond = 1:12 (3). NBS, *N*-bromosuccinimide; *T/I*, ratio of terminal (*T*) double bonds to internal (*I*) double bonds; DCM, dichloromethane.

TABLE 2
Regioselective Oxidation of Squalene Using Different Epoxidation Procedures

	Catalyst/oxidant	Solvent	X (%)	S_{internal} (%)	S_{terminal} (%)	Molar ratio T/I
	Venturello and D'Aloisio (9)					
1	PW$_4$-Trioctylmethylammonium/H$_2$O$_2$	DCM[a]	21	60	40	3.0
2	PW$_4$-Trioctylmethylammonium/H$_2$O$_2$	DCM	10	58	42	2.8
3	Hexadecyltrimethylammonium/H$_2$O$_2$	DCM	6.5	50	50	2.0
4	PW$_4$-Dodecyldimethylephidriniumbromide/H$_2$O$_2$	DCM	11	45	55	1.6
5	PW$_4$-Benzyldimethylhexadecylammonium/H$_2$O$_2$	DCM	7	52	48	2.2
6	PW$_4$-Benyldimethyltetradecylammonium/H$_2$O$_2$	DCM	13	53	47	2.3
	Dioxirane chemistry					
7	Oxone	Acetone	6	64	36	3.8
	Payne chemistry					
8	H$_2$O$_2$	MeOH, H$_2$O, and CH$_3$CN	1	47	53	1.8
	Ti-MCM-41					
9	Ti-MCM-41/H$_2$O$_2$	Decane/toluene	16	50	50	2

[a] Molar ratio oxidant/double bond of 1:6 is used. For abbreviation see Table 1.

ratio of 1.6. Dioxirane, which is formed upon reacting oxone with acetone, preferentially epoxidizes the terminal double bonds (Table 2, entry 7). In contrast, Payne chemistry (Table 2, entry 8) and classic metal-catalyzed peroxide chemistry [e.g., with the heterogeneous Ti-MCM-41 catalysts (14), entry 8] give lower *T/I* ratios, e.g., 1.8 and 2, respectively.

In summary, we have found a straightforward method for the fast and accurate quantitative determination of the internal and terminal epoxides of squalene using ^{1}H NMR. The reliability of the NMR method is evaluated by comparison with the regioselectivities determined using the old analysis. This new method will facilitate further research in understanding the regioselective epoxidation of squalene during steroid synthesis and of other analog linear terpenes (e.g., farnesyl alcohols).

ACKNOWLEDGMENTS

H.A.J.A. acknowledges sponsoring by N.V. Oleon. B.F.S. is indebted to FWO (Fonds voor Wetenschappelijk Onderzoek, Belgium) for a postdoctoral fellowship. This work is supported by the Federal Government of Belgium in the frame of an IAP (Interuniversitaire Attractie Pool) project on Supramolecular Chemistry and Catalysis.

REFERENCES

1. Aerts, H.A.J., and P.A. Jacobs, Epoxide Yield Determination of Oils and Fatty Acid Methyl Esters Using ^{1}H NMR, *J. Am. Oil Chem. Soc. 81*:841–846 (2004).
2. Wendt, K.U., G.E. Schulz, E.J. Corey, and D.R. Liu, Enzyme Mechanisms for Polycyclic Triterpene Formation, *Angew. Chem. Int. Ed. 39*:2812–2833 (2000).
3. Bai, M., X. Xiao, and G.D. Prestwich, Epoxidation of 2,3-Oxidosqualene to 2,3;22,23-Squalene Dioxide by Squalene Epoxidase, *Biochem. Biophys. Res. Commun. 185*:323–329 (1992).
4. Van Tamelen, E.E., and T.J. Curphey, The Selective *in vitro* Oxidation of the Terminal Double Bonds in Squalene, *Tetrahedron Lett. 3*:121–124 (1962).
5. Van Tamelen, E.E., and K.B. Sharpless, Positional Selectivity During Controlled Oxidation of Polyolefins, *Ibid. 8*:2655–2659 (1967).
6. Van Dommelen, M.E., A.R.N. Wilson, J.W. de Haan, and H.M. Buck, A ^{13}C-NMR Study of Squalene. Some Aspects of the Selective Terminal Oxidation of Squalene, *J. R. Neth. Chem. Soc. 94*:206–208 (1975).
7. Pogliani, L., M. Ceruti, G. Ricchiardi, and D. Viterbo, An NMR and Molecular Mechanics Study of Squalene and Squalene Derivatives, *Chem. Phys. Lipids 70*:21–34 (1994).
8. Abad, J.-L., J. Casas, F. Sánchez-Baeza, and A. Messeguer, Dioxidosqualenes: Characterization and Activity as Inhibitors of 2,3-Oxidosqualene-Lanosterol Cyclase, *J. Org. Chem. 58*:3991–3997 (1993).
9. Venturello, C., and R. D'Aloisio, Quaternary Ammonium Tetrakis(diperoxotungsto)phosphates (3−) as a New Class of Catalysts for Efficient Alkene Epoxidation with Hydrogen Peroxide, *J. Org. Chem. 53*:1553–1557 (1988).
10. Grün, M., I. Lauer, and K.K. Unger, The Synthesis of Micrometer- and Submicrometer-Size Spheres of Ordered Mesoporous Oxide MCM-41, *Adv. Mater. 9*:254–257 (1997).
11. Franke, O., J. Rathousky, G. Schulz-Ekloff, J. Stàrek, and A. Zukal, New Mesoporous Titanosilicate Molecular Sieve, *Stud. Surf. Sci. Catal. 84*:77–84 (1994).
12. Sonnet, P.E., M.E. Lankin, and G.P. McNeill, Reactions of Dioxiranes with Selected Oleochemicals, *J. Am.Oil Chem. Soc. 72*:199–204 (1995).
13. Epoxides, 3.2 Epoxidation with Hydrogen Peroxide, in *Ullmann's Encyclopedia*, 6th edn., 2000 Electronic Release.
14. deVilla, P.A., B.F. Sels, D.E. De Vos, and P.A. Jacobs, A Heterogeneous Tungsten Catalyst for Epoxidation of Terpenes and Tungsten-Catalyzed Synthesis of Acid-Sensitive Terpene Epoxides, *J. Org. Chem. 64*:7267–7270 (1999).

[Received November 17, 2004; accepted May 9, 2005]