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Chemical Evidence for the Participation of a Perepoxide Intermediate in the Reaction of Singlet Oxygen with Mono-olefins in Relationship with the Biogenetic Pathway to Highly Oxidized Abietane Diterpenes

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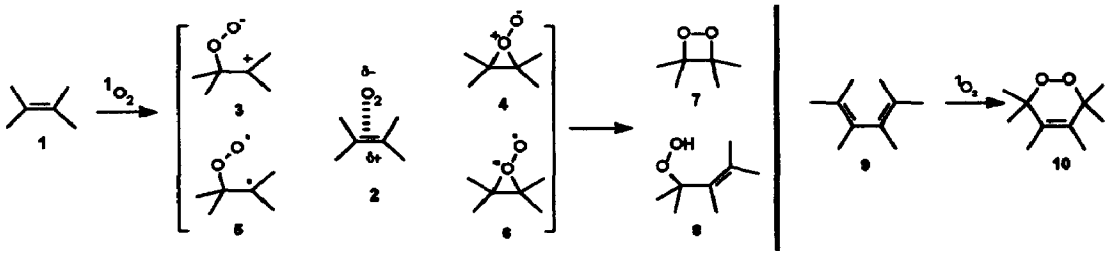
Abstract: **Chemical evidence has been obtained in two separate experiments for the participation of a perepoxide intermediate in the reaction of singlet oxygen with mono-olefins. The intramolecular trapping of such an intermediate in the reaction of 6,7-didehydrocarnosic acid derivatives with molecular oxygen in unequivocal singlet oxygen-generating conditions to give rosmanol and isorosmanol derivatives represents conclusive proof for the cited mechanism. Futhermore, such chemical evidence supports our earlier hypothesis of a biogenetic pathway to highly oxidized abietatriene diterpenes in which enzymatic dehydrogenation and singlet-state oxygen appear to play important roles.**

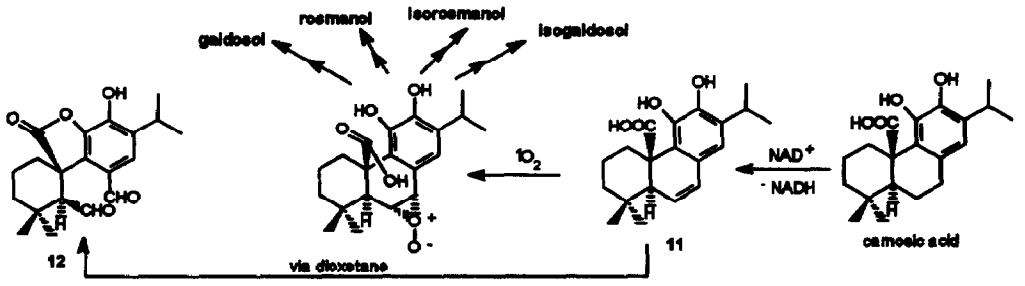
Oxygen, in its singlet state, is a versatile reagent[1]. Depending on the availability of an allylic hydrogen substituent, mono-olefins **1** react with it to give either dioxetanes **7** or hydroperoxides **8** , whereas cis-dienes **9** yield endo-peroxides **10** (Scheme 1). The mechanism of these reactions has received considerable attention in recent years. Although it is generally conceded that the last reaction proceeds through a concerted [4+2] cycloaddition, the mechanisms of the first and second reactions remain controversial. Concerted [2+2] additions and ene-type reactions are attractive for their mechanistic economy[2] but two-step processes are more likely. As a first event, oxygen may interact with olefins **1** to give exciplexes **2**[3], zwitterionic peroxides **3** or perepoxides **4**[4], or their biradical analogues **5** and **6**[5,6]. Subsequent skeletal or electronic reorganization of these different intermediates generates the appropriate hydroperoxide or dioxetane (Scheme 1). Since none of these intermediates have been isolated, they were deemed to be unstable. Consequently, evidence for their existence has been difficult to obtain and necessarily has been of the indirect kind. Recently it has been demonstrated that the dye-sensitized photo-oxygenation of trisubstituted olefins of at least the norbornene type passes through a zwitterionic peroxide[7], which is sufficiently long-lived to be trapped by alcohols and aldehydes.



SCHEME 1

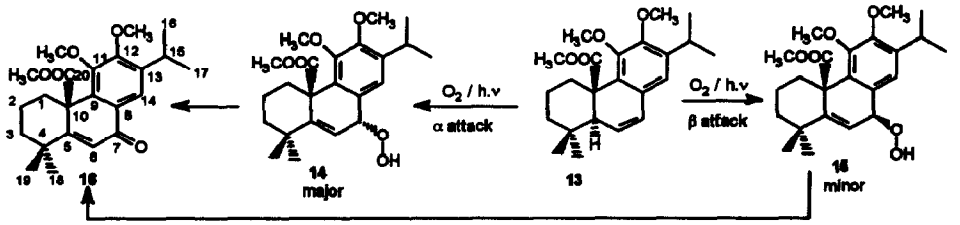
In relationship with our previously reported postulation[8] of a biogenetic pathway to lactonic abietatriene diterpenes such as rosmadial **12**, galdosol , isogaldosol , rosmanol , isorosmanol (Scheme 2) and to other highly oxidized abietanic diterpenes and diterpenequinones,[9-13] in *Salvia* species, in which enzymatic dehydrogenation processes and the participation of singlet-state oxygen appear to play an important role and also with the

possible participation of this type of compounds in defensive mechanisms protecting the plant cells of *Salvia* species against injuries by free radicals and singlet oxygen, we now present an account of experiments in which perepoxide intermediates have been characterized by intramolecular trapping experiments.



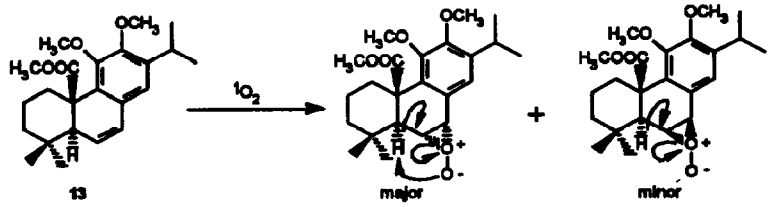
SCHEME 2

As shown in Scheme 2, 6,7-didehydrocarnosic acid **11** plays a key role in our hypothesized biogenetic pathway. When 6,7-didehydrocarnosic acid methyl ester dimethylether **13** (Scheme 3), which we have isolated as a natural product[14] , was left in air in a solution of acetone-water, it underwent a slow and interesting oxidation which after 15 days had resulted in its partial transformation into enone **16**, which had physical and spectroscopic data superimposable on those of an authentic sample[15]. In this process hydroperoxides **14** (major) and **15** (minor) were isolated and characterized[16] as intermediates.



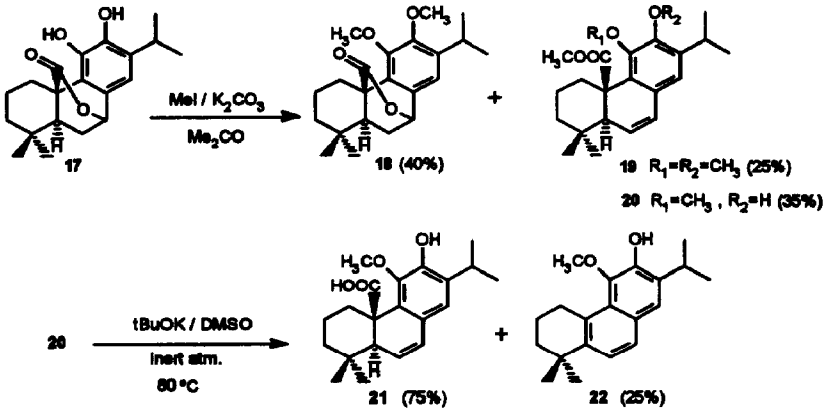
SCHEME 3

The formation of both epimeric hydroperoxides, with the α-epimer as the major one (more stereochemically hidden β face), appears, in principle, to indicate a radical type process via formation of a benzylic radical on C-7. However, in this and in repeated experiments the above hydroperoxides were formed with 100% allylic transposition and no hydroperoxide on C-5 was detected in any case. On the other hand, when the above reaction (also over 15 days) was carried out adding sodium azide, an agent which is known to inhibit singlet oxygen reactions[17], no reaction occurred and unaltered starting material was recovered, precluding the participation of radical type species. The participation of singlet-state oxygen in the above reaction, via a concerted "ene" type reaction and thus a stereospecific process, is not compatible with the formation of the β-hydroperoxide **15**. Nevertheless, all the experimental features of the above reaction may be accounted for by the participation of a perepoxide intermediate as indicated in Scheme 4. On the other hand, substrates such as 6,7-didehydrocarnosic acid **11** or derivatives thereof with a free carboxylic acid group appear to possess the suitable structural features to attempt to prove the possible participation of a perepoxide intermediate, in the reaction of the C_6-C_7 double bond with singlet-state oxygen, by intramolecular trapping of the perepoxide by the free carboxylic group. This led us to attempt to prove our hypothesis in the laboratory.



SCHEME 4

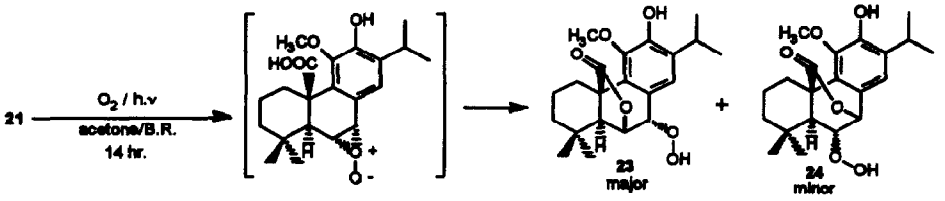
6,7-Didehydrocarnosic acid 11-methylether **21** was obtained as indicated in Scheme 5. Treatment of carnosol **17** (789 mg) disolved in dry acetone (80 ml) with methyl iodide (10 ml) and potassium carbonate (10 mg) in inert atmosphere and reflux for 24 hrs gave a mixture of 11,12-di-O-methyl carnosol **18**, 6,7-didehydrocarnosic acid methyl ester dimethylether **19** and 6,7-didehydrocarnosic acid methyl ester 11-methylether **20**, which were separated by preparative TLC. The spectral data of **18** and **19** were superimposable on those of authentic samples[18,15]. The structure of **20**[19] was confirmed from its spectroscopic data and the free phenolic group was situated on C-11 on the basis of the strong nOe effect observed between the proton of the phenolic group and the methyl groups of the isopropyl grouping in a NOEDIFF experiment.



SCHEME 5

Hydrolysis of **20** (126.1 mg) with potassium t-butoxide (300 mg) in dimethylsulfoxide (50 ml) at 80°C in inert atmosphere for 24 hrs yielded 6,7-didehydrocarnosic acid 11-methylether **21** as the major product together with traces of the decarboxylation product **22**[20]. When an ice-cold bath solution of **21** (6.5 mg) in tridistilled $CHCl_3$ (30ml), with Bengal Rose added, placed in an assay tube, was bubbled with molecular oxygen and illuminated with an intense beam of visible light (500w spotlight) it gave after 14hrs a mixture of two products (Scheme 6) which were separated by preparative TLC and characterized by their spectral data[21] as 11-methylether-7-hydroperoxide of rosmanol **23** and 11-methylether-6-hydroperoxide of isorosmanol **24**. The latter was a minor product, which shows in its ^{1}HNMR spectrum signals for H-6 and H-7 protons with multiplicities and chemical shifts identical with those found for the corresponding protons in the ^{1}HNMR spectrum of isorosmanol[22]. The ^{1}HNMR spectrum of the major product **23** was identical with that of an authentic sample of 11-methylether of rosmanol obtained by treatment of rosmanol with diazomethane in ether (excepting the chemical shift of the H-14 aromatic proton, wich appear at δ 7.01 in the late and at δ 6.87 in that of **23**). On the other hand, in the mass spectrum of **23**, in which the ion molecular was not observed, the main peacks are those corresponding to lost from it of an oxygen atom or of H_2O and derived from them. The above

reaction constitutes clear evidence of the participation of a perepoxide intermediate in the interaction of mono-olefins with singlet oxygen.



SCHEME 6

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REFERENCES AND NOTES

1. (a) Schaap, A.P., ed., *Singlet molecular oxygen;* Dowden, Hutchinson and Ross. Inc., Stroudsburg, PA, 1976. (b) Wasserman, H. H. and Murray, R. W., ed., *Singlet oxygen;* Academic Press. New York. NY. 1979. (c) Frimer, A. A., *Singlet oxygen;* Vol 2, CRC Press Inc., Boca Raton, FL, 1985.
2. Frimer, A. A. *Chem. Rev.*, **1979**, *79*, 359.
3. (a) Gorman, A. A.; Gould, I. R.; Hamblett, I. *J. Am. Chem. Soc.*, **1982**, *104*, 7098. (b) Gorman, A. A.; Hamblett, I.; Lambert, C.; Spencer, B.; Standen, C. *J. Am. Chem. Soc.* **1988**, *110*, 8053.
4. (a) Foote, S.; Mazur,S.; Burns, P. A.; Lerdal, D. *J. Am. Chem. Soc.*, **1973**, *95*, 586. (b) Takeshita, H., Hatsui, T.; Jinnai, O. *Chem. Lett.*, **1976**, 1059.
5. Yamaguchi, K.; Yabushita, S.; Fueno, T.; Houk, K. N. *J. Am. Chem. Soc.*, **1981**, *103*, 5043.
6. Enke, Von F., ed. *Die Ketene;* Stuttgart, Vol1, 1912, p 55.
7. Jefford, W. *Chem. Soc. Rev.*, **1993**, 59.
8. González, A. G. ; Aguiar, Z. E. ; Grillo, T. A.; Luis, J. G.; *Phytochemistry*, **1992**, *31*, 1691.
9. González, A. G.; Castro, Z. E. A.; Luis, J. G.; Ravelo, A. G. *J. Chem. Res. (s)*, **1989**, 132.
10. González, A. G.; Aguiar, Z. E.; Luis, J. G.; Ravelo, A. G. *Tetrahedron*, **1989**, *45*, 5203.
11. Luis, J. G.; Grillo, T. A. *Tetrahedron*, **1993**, *49*, 6277.
12. González, A. G.; S. Andrés, L.; Aguiar, Z. E.; Luis, J. G. *Phytochemistry*, **1992**, *31*,1297.
13. Luis, J. G.; S. Andrés, L.; Perales, A. *Tetrahedron*, **1993**, *49*, 4993.
14. González, A. G.; Rodríguez, C. M.; Luis, J. G. *Phytochemistry*, **1987**, *26*, 1471.
15. González, A. G.; Rodríguez, C. M.; Luis, J. G. *J. Chem. Res. (s)*, **1988**, 114.
16. Compound **14** IR ν_{max} ($CHCl_3$) cm^{-1}: 3640, 3010, 3000, 2950, 2920, 2660, 1720, 1600, 1460, 1445, 1325, 1280, 1220, 1100, 1070, 1040, 1000, 980. EM m/z (%): 386 [M^+-H_2O] (23), 371 (11), 328 (37), 327 (100), 313 (14), 306 (13), 297 (13), 285 (11), 279 (10), 259 (10), 243 (19), 201 (9), 167 (14), 149 (80), 113 (18). ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.08, 1.27 (each 3H, s, Me-18 and Me-19), 1.26 (6H, d, J= 7.0 Hz, Me-16 and Me-17), 3.31 (2H, overlap signal, H-1β, H-15), 3.58 (3H, s, $COOCH_3$), 3.71 (3H, s, Ar-OCH_3), 3.78 (3H, s, Ar-OCH_3), 5.47 (1H, d, J=3.3 Hz, H-6), 6.13 (1H, d, J=3.3 Hz, H-7), 7.19 (1H, s, H-14), 7.47 (1H, s, -O-OH).
Compound **15** ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.09-1.27 [4 Me], 5.62 (1H, d, J= 3.3 Hz, H-6), 6.28 (1H, d, J= 3.3 Hz, H-7), 7.12 (1H, s, -O-OH), 7.29 (1H, s, H-14).
17. Yamamoto, Y.; Furuta, T.; Matsuo, J.; Kurata, T. *J. Org. Chem.*, **1991**, *56*, 5737.
18. Fraga, B. M.; González, A. G.; Herrera, J. R.; Luis, J. G.; Ravelo, A. G. *Phytochemistry*, **1986**, *25*, 269.
19. Compound **20** ^1H NMR ($CDCl_3$, 200 MHz) δ: 0.88 (3H, s, Me-19), 1.03 (3H, s, Me-18), 1.19, 1.22 (each 3H, d, J= 7.0 Hz, Me-16 and Me-17), 2.59 (1H, t, J=3.0 Hz, H-5), 3.19 (1H, hept, J=7.0 Hz, H-15), 3.54 (3H, s, $COOCH_3$), 3.76 (3H, s, Ar-OCH_3) , 6.06 (1H, dd, J_1=2.8 Hz, J_2=9.6 Hz, H-6), 6.20 (1H, s, Ar-OH), 6.35 (1H, dd, J_1= 2.8 Hz, J_2= 9.6 Hz, H-7), 6.51 (1H, s, H-14).
20. Compound **21** ^1H NMR ($CDCl_3$, 200 MHz) δ: 0.96 (3H, s, Me-19), 1.02 (3H, s, Me-18), 1.19, 1.20 (each 3H, d, J= 7.0 Hz, Me-16 and Me-17), 2.60 (1H, d, J=5.1 Hz, H-5), 3.19 (1H, hept, J=7.0Hz, H-15), 3.75 (3H, s, Ar-OCH_3), 6.09 (1H, dd, J_1=3.8 Hz, J_2=10.0 Hz, H-6), 6.39 (1H, dd, J_1= 2.1 Hz, J_2= 10.0 Hz, H-7), 6.45 (1H, s, Ar-OH), 6.52 (1H, s, H-14).
Compound **22** EM m/z (%): 298 (70), 283 (100), 268 (14), 255 (10), 254 (20), 237 (19), 223 (32), 207 (23), 178 (32), 165 (55), 115 (15). ^1HNMR ($CDCl_3$, 200 MHz) δ: 1.30 (6H, d, J=7.0 Hz, Me-16 and Me-17), 1.36 (6H, s, Me-19 and Me-18), 3.36 (3H, overlap signal, H-15, H-1), 3.73 (3H, s, Ar-OCH_3), 6.20 (1H, bes, Ar-OH), 7.28 (1H, d, J= 8.6 Hz, H-6), 7.35 (1H, s, H-14), 7.52 (1H, d, J=8.6 Hz, H-7).
21. Compound **23** EM m/z (%) : 360 [M^+- O] (34), 358 [M^+-H_2O] (9), 346 [M^+- (2xCH_2)] (4), 330 [M^+- O- (2xCH_2)] (4), 328 [M^+-H_2O- (2xCH_2)] (4),316 (8), 314 [M^+-H_2O-CO_2] (9), 301 (29), 299 (20), 287 (20), 284 (4), 231 (35), 177 (23), 149 (100). ^1H NMR ($CDCl_3$, 200 MHz) δ: 0.95 (3H, s, Me-19), 1.01 (3H, s, Me-18), 1.21, 1.23 (each 3H, d, J= 7.0 Hz, Me-16 and Me-17), 2.20 (1H, s, H-5), 3.20 (1H, hept, J= 7.0 Hz, H-15), 3.24 (1H, brd, H-1β), 3.76 (3H, s, Ar-OCH_3), 4.58 (1H, d, J=3.3 Hz, H-6), 4.76 (1H, d, J=3.3 Hz, H-7), 6.13 (1H, s, Ar-OH), 6.87 (1H, s, H-14).
Compound **24** ^1H NMR ($CDCl_3$, 200 MHz) δ: 0.95 (3H, s, Me-19), 1.05 (3H, s, Me-18), 1.20, 1.22 (each 3H, d, J=7.0 Hz, Me-16 and Me-17), 1.31 (1H, d, J=3.8 Hz, H-5), 2.78 (1H, brd, H-1β), 3.32 (1H, hept, J= 7.0 Hz, H-15), 3.76 (3H, s, Ar-OCH_3), 4.37 (1H, t, J= 4.6 Hz, H-6), 5.25 (1H, d, J= 4.6 Hz, H-7), 6.60 (1H, s, Ar-OH), 7.10 (1H, s, H-14).
22. González, A. G.; S. Andrés, L.; Luis, J. G.; Brito, I.; Rodríguez, M. L. *Phytochemistry*, **1991**, *30*, 4067.

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