containing 5 mg napthaleneacetic acid and 0.1 mg kinetin per l. Cell suspensions were obtained from calluses in the same medium lacking agar (50 ml medium in 200 ml Erlenmeyer flasks, incubated at 25° on a rotary shaker) and routinely transferred to fresh medium every 7 days. ^{14}C-Labelled capsidiol, rishitin and lubimin, available from previous studies [1, 6], were used initially to facilitate recognition of the products on chromatograms. Non-radioactive starting materials were used subsequently, and all available materials were bulked for isolation. Procedures for GLC have been described [7]. Si gel was used for both TLC and CC. 13-Hydroxycapsidiol (1, R = OH) was isolated by PLC (*t*-BuOH–EtOAc–HOAc, (5:95:0.5); $R_{capsidiol}$ *ca* 0.6) and purified by CC in *iso*-PrOH–EtOAc. (1:9). The product (5.5 mg from 5 l. diffusates) crystallized from $CHCl_3$ on cooling: colourless crystals containing solvent (typical $CHCl_3$ peaks in MS), mp indistinct at 85–95°. MS *m/e* (rel. int.): 252 (< 1, M^+) 237 (18, M—Me), 234 (8, M—H_2O), 219 (10, M—Me—H_2O), 216 (32, M—$2H_2O$), 201 (24, M—Me—$2H_2O$), 198 (17, M—$3H_2O$), 183 (28, M—Me—$3H_2O$), 105 (100). Precise mass measurements: calculated for $C_{15}H_{24}O_3$—Me, 237.1491: found, 237.1498; calculated for $C_{15}H_{24}O_3$—H_2O, 234.1620; found 234.1622. PMR (CD_3CN) δ: 5.84 (*q*, 1H, 9-H), 4.82 (*m*, 1H), 4.99 (*m*, 1H) (12-H's), 4.01 (*bs*, 2H, 13-H's), 4.21 (*dd*, 1H, 1-H), 4.41 (*d* of *t*, 1H, 3-H), 1.31 (*s*, 3H, 14-Me), 0.82 (*d*, 3H, 15-Me); (CD_3OD) δ: 5.85 (*q*, 1H, 9-H), 4.99 (*m*, 1H), 4.79 (*m*, 1H) (12-H's), 4.40 (*d* of *t*, 1H, 3-H), 4.22 (*dd*, 1H, 1-H), 4.01 (*bs*, 2H, 13-H's), 1.32 (*s*, 3H, 14-Me), 0.85 (*d*, 3H, 15-Me); ^{13}C-NMR (CD_3OD) δ: 154.7 (C-11), 108.5 (C-12), 129.3 (C-9), 141.4 (C-10), 75.6 (C-1), 66.0 (C-3), 65.4 (C-13), 49.0 (C-4), 47.1 (C-6), 40.3 (C-5), 37.1 (C-7), 37.1 (C-2), 31.9 (C-8), 32.5 (C-14), 9.6 (C-15). Material (6.1 mg) similarly isolated and crystallized from pepper tissue cultures (4 l.) was identical with the compound from fruit diffusates by TLC, mp, PMR, and MS. Acetylation (Ac_2O–Py at room temp.) gave the triacetate, whose PMR contained the requisite three acetate methyl signals at 1.98, 2.00 and 2.03 ppm. MS *m/e* (rel. int.): 336 (2, M—C_2H_2O), 318 (4, M—$C_2H_4O_2$), 276 (22, M—$C_2H_4O_2$—C_2H_2O), 258 (100, M—$2C_2H_4O_2$), 216 (42, M—$2C_2H_4O_2$—C_2H_2O), 198 (81, M—$3C_2H_4O_2$), and 183 (27, M—$3C_2H_4O_2$—CH_3); precise mass, calculated for $C_{21}H_{30}O_6$—$2C_2H_4O_2$, 258.1620; found, 258.1621.

13-Hydroxyrishitin. The ether-extracted material from potato tissue cultures (total, 3.5 l.) was chromatographed (5 ml fractions) first over Si gel (65 g) in MeOH–$CHCl_3$ (5:95), and again over Si gel (40 g) in *iso*-PrOH–EtOAc (1:9). Evapn of the only major radioactive band (fractions 19–23, 2nd system) furnished chromatographically almost homogeneous 13-hydroxyrishitin (2, R = OH) as a syrup (3.8 mg); PMR (CD_3OD) δ: 4.97, 4.74 (*m*, 1H each, 12-H's), 3.96 (*bs*, 2H, 13-H's) 3.38 (*m*, 1H, 2-H), 3.30 (*dd*, 1H, 3-H), 1.05 (*d*, 3H, 15-H's); ^{13}C-NMR (CD_3OD) δ: 153.5 (C-11), 108.8 (C-12), 130.2 (C-5), 125.9 (C-10), 79.8 (C-3), 72.0 (C-2), 65.2 (C-13), 43.1 (C-4), 39.2 (C-1), 37.0 (C-7), 32.7 (C-6), 30.2 (C-9), 27.7 (C-8), 17.0 (C-15); MS *m/e* (rel. int.): 238 (7, M^+), 220 (60, M—H_2O), 205 (14, M—H_2O—Me), 202 (24, M—$2H_2O$), 187 (34, M—$2H_2O$—Me), 184 (11, M—$3H_2O$), and 91 (100); precise mass, calculated for $C_{14}H_{22}O_3$, 238.1569; found, 238.1568. Additional peaks in the MS at *m/e* 278 (7), 236 (3, 278—C_2H_2O), and 218 (7, 278—$C_2H_4O_2$) suggested that the sample contained a little monoacetate as an impurity but in too low a concentration to be detectable in the NMR spectrum. Acetylation of the substance (2 mg) furnished the triacetate, which exhibited three 3-proton singlets at 2.01, 2.05 and 2.08 ppm as clear proof of triacetylation. MS *m/e* (rel. int.): (1.2, M—$C_2H_4O_2$), 262 (1.8, M—$C_2H_4O_2$—C_2H_2O), 244 (4.0, M—$2C_2H_4O_2$), 202 (7.0, M—$2C_2H_4O_2$—C_2H_2O), 184 (86, M—$3C_2H_4O_2$), 169 (31, M—$3C_2H_4O_2$—Me), and 143 (100); precise mass, calculated for $C_{20}H_{28}O_6$—$C_2H_4O_2$, 304.1674; found 304.1679.

Acknowledgements—We wish to thank B. Ollerenshaw and M. Brown for technical assistance and H. Schroeder and D. Hairsine for PMR and MS respectively.

REFERENCES

1. Stoessl, A., Robinson, J. R., Rock, G. L. and Ward, E. W. B. (1977) *Phytopathology* **67**, 64.
2. Gordon, M., Stoessl, A. and Stothers, J. B. (1973) *Can. J. Chem.* **51**, 748.
3. Birnbaum, G. I., Stoessl, A., Grover, S. H. and Stothers, J. B. (1974) *Can. J. Chem.* **52**, 993.
4. Anderson, R. C., Gunn, D. M., Murray-Rust, J., Murray-Rust, P. and Roberts, J. S. (1977) *J. Chem. Soc. Chem. Commun.* 27.
5. Murashige, T. and Skoog, F. (1963) *Physiol. Plantarum* **15**, 473.
6. Stoessl, A., Ward, E. W. B. and Stothers, J. B. (1976) *Tetrahedron Letters* **37**, 3271.
7. Ward, E. W. B. and Stoessl, A. (1977) *Phytopathology* **67**, 468.

Phytochemistry, 1977, Vol. 16, pp. 2025–2027 Pergamon Press. Printed in England.

THE CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRA OF FOUR EUDESMANE SESQUITERPENOLS

NANCY N. GERBER and DOROTHY Z. DENNEY

Institute of Microbiology and School of Chemistry Rutgers University—The State University of New Jersey, P.O. Box 759, Piscataway, NJ08854, U.S.A.

(*Received* 27 *May* 1977)

Key Word Index—*Streptomyces*; sesquiterpenols; ^{13}C NMR; dihydroeudesmol, geosmin.

Abstract—The ^{13}C NMR spectra of geosmin, selina-4(14),7(11)-diene-99-ol and two dihydroeudesmol isomers have been obtained and the individual resonances assigned. Several different empirical correlations developed by others have been combined in simple calculations to predict chemical shift values for sesquiterpenols of the eudesmane group.

INTRODUCTION

With the availability of pulsed-Fourier transform ^{13}C NMR spectroscopy it became of interest to see how useful this physical method might be in the structure

Table 1. Calculated chemical shift values for the 4,10-dimethyl, 4,7,10-trimethyl and 4,10-dimethyl-7-isopropyl decalin frames*

Carbon	4,10 Dimethyl	4,7,10-Trimethyl	4,10-Dimethyl-8-isopropyl†
1	42.4	42.4	42.4
2	22.2	22.2	22.2
3	37.3	37.3	37.3
4	33.1	33.1	33.1
5	52.6	52.6	52.6
6	25.7	34.3	29.0
7	27.4	33.3	44.0
8	22.2	30.7	25.4
9	42.4	42.4	42.4
10	34.8	34.8	34.8

*In ppm relative to TMS. In the calculations substitution parameters of less than one ppm were omitted.

†These values are close to those observed for the aliphatic carbons of β-selinene [8]. The average difference between the calculated and observed values is 1.3 ppm.

elucidation of sesquiterpenoids. It was not feasible to obtain many different pure sesquiterpenoids of known structure and determine their ^{13}C NMR spectra as has been done for monoterpenes [1] and steroids [2]. Therefore we selected and used in simple calculations several different empirical correlations which have been developed by others [2,3] for the relationship between structure and chemical shift. The generally good agreement between our calculated and observed chemical shift values for several sesquiterpenols of the eudesmane group prompt us to report our findings.

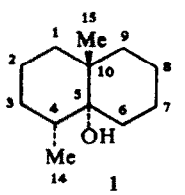
RESULTS AND DISCUSSION

Comparing the reported chemical shift values for *trans*-decalin and *trans-anti*-1-methyldecalin [3] one can obtain methyl substituent parameters for this substitution. These parameters applied to the known values for *trans*-10-methyldecalin [3], enables one to calculate chemical shift values for the *trans*-4,10-dimethyl-*trans*-decalin frame (Table 1). Then, using this frame and the α, β and γ substituent effects which were developed from a study of 31 monohydroxylated steroids [2] one can calculate chemical shift values for geosmin (1) [4]. The calculated and observed values are compared in Table 2. There are no skew pentane interactions; there are 5γ-gauche interactions. between the hydroxy group and carbons 1,3,7,9 and 14. The constant terms for tertiary alcohols were used and the average value of −6 ppm for the *γ-gauche* substituent effect on methylene carbons.

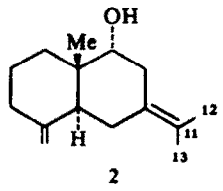
In exactly the same way one can obtain methyl substituent parameters by comparing *trans*-decalin and *trans*-syn-2-methyldecalin [3]. These parameters applied to the *trans*-4,10-dimethyl-*trans*-decalin frame enable one to calculate chemical shift values for the 4,7,10-trimethyl decalin frame (Table 1). Then, comparing the values for methyl and *t*-butyl substituted 1-methyl cyclohexanols [5] one can derive numbers for the replacement of methyl by *t*-butyl (+ 16 ppm for the α carbon, −8 ppm for both β carbons). Using these numbers one can calculate the 4,10-dimethyl-7-*t*-butyl frame (Table 2 footnote **) and using 2/3 of these values one can calculate values for the 4,10-dimethyl-7-isopropyl decalin frame (Table 1).

The 4,10-dimethyl-7-*t*-butyl decalin frame is in excellent agreement with the observed chemical shifts of the 4-α-methyldihydroeudesmol (3). Comparing the chemical shifts of 4α- and 4β-hydroxysteroids (Table 3 in ref.[2]) one can derive reasonable chemical shift values for the 4β-methyldihydroeudesmol isomer 4. Both calculated and observed values are shown in Table 2.

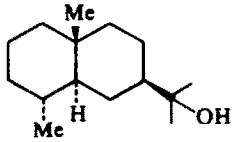
Using the 4,10-dimethyl-7-isopropyl decalin frame and the equations derived from hydroxysteroids [2] one can calculate values for the aliphatic carbons of



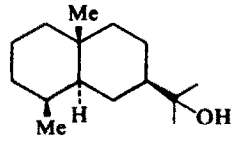
1



2



3



4

Table 2. Calculated and observed chemical shifts of four eudesmane sesquiterpenols*

Carbon	1		2		3		4	
	calc.	obs.	calc.	obs.	calc**.	obs.	calc.	obs.
1	36.4	34.9*t*†	36.4	36.8*t*†	42.4	41.9*t*†	42.4	44.6*t*†
2	22.2	20.6*t*	22.2	23.3*t*	22.2	21.6*t*§	18.7	17.4*t*
3	31.3	30.3*t*§	37.3	37.3*t*†	37.3	36.7*t*	34.9	33.6*t*‡
4	36.0	34.1*d*	—	149.5*s*	33.1	31.5*d*	35.1	33.7*d*‡
5	75.1	74.3*s*	52.6	48.3*d*	52.6	51.3*d*	48.5	47.0*d*
6	31.0	29.7*t*§	29.0	27.9*t*	26.3	24.9*t*	29.6	28.0*t*
7	21.4	21.2*t*‡	—	126.9*s*	49.3	49.6*d*	49.3	50.1*d*
8	22.2	20.6*t*‡	37.7	34.6*t*	22.7	22.3*t*†	22.7	22.7*t*
9	36.4	35.5*t*†	80.4	79.6*d*	42.4	41.7*t*§	42.4	41.6*t*†
10	37.7	37.1*s*	39.3	41.2*s*	34.6	33.4*s*	33.3	33.6††
11				123.1*s*		73.0*s*		72.8*s*
12				20.2*q*		21.8*q*‡		26.9*q*§
13				20.2*q*		27.2*q*‡		27.1*q*§
14	13.7¶	14.7*q*‡‡		106.9*t*		20.1*q*‖		19.5*q*‖
15		20.1*q*‡‡	9.6¶	10.1*q*		16.7*q*‖		14.8*q*‖

*In parts per million relative to TMS. The average difference between calculated and observed values is 1.1 for 1 and 2, 0.9 for 3 and 1.25 for 4. *q*, *t*, *d*,*s* describes the appearance of each band in the single frequency off-resonance decoupling experiment and hence the degree of substitution.

†‡§Numerical values with identical superscripts may be exchanged.

‖Assigned by analogy to the methyl values for *trans*-10-methyldecalin (15.6 ppm) and *trans*-anti-1-methyldecalin (19.7 ppm) given in ref. [3].

¶This calculation uses a −6 ppm value for the *γ-gauche* interaction of methyl with the hydroxy group, in accord with ref. [2].

**These are the calculated values for the 4,10-dimethyl-7-*t*-butyldecalin frame.

††A distinct line for C-10 was not observed. Normally the line for this tetra substituted carbon is 1/3–1/2 as tall as the others. It was assumed to be at 33.6 ppm and obscured by the two stronger lines in this region.

‡‡This is in agreement with the methyl values for cybullol [9], a hydroxygesmin. The observed CMR values for the ring carbons of cybullol [9] were in good agreement (average difference 1 ppm) with values calculated by the methods described here.

selina-4(14), 7(11)-diene-9-ol [6]. The stereochemistry of **2** was shown by its circular dichroism curve which was very similar to that of authentic 10-β-methylselina-4(14),7(11)-diene (Anderson, N. H., personal communication). The reasonably good agreement between the calculated and observed values for **2** indicate that the exocyclic double bonds do not seriously alter the shape of the *trans*-decalin system.

EXPERIMENTAL

Geosmin (**1**) and the selinadienol (**2**) were pure substances previously isolated from *Streptomyces* [4,6]. The dihydroeudesmol mixture [7] was a gift from B. Maurer. The dihydroeudesmol isomers, **3** and **4**, were separated by GLC in 2 mg amounts on a 1.9 m by 5 mm column of 10% SE-30 on diatoport W 60–80 mesh operated isothermally at 180°. The faster eluting, less abundant isomer was **3**; the slower eluting more abundant one was **4**.

The ^{13}C NMR spectra were recorded at 20.0 MHz using a Varain C FT-20 spectrometer. The data were acquired by pulsing the sample for about 12 hr at a 40° flip angle, which corresponds to a 7 μsec pulse, and with an aquisition time of 1.023 sec. The chemical shifts are relative to internal TMS and are estimated to be accurate to ± 0.2 ppm. The ^{13}C spectra were first recorded in the proton noise-decoupled mode in order to measure the exact chemical shifts of all of the ^{13}C nuclei. In further series of experiments the degree of substitution of each nucleus was determined by running single frequency off-resonance decoupling experiments. The spectra were determined on 10 to 30 mg samples dissolved in 1 ml $CDCl_3$.

Acknowledgements—We thank B. Maurer, Firmenich, Geneva, for a generous gift of the dihydroeudesmol mixture and N. H. Anderson, University of Washington, for the circular dichroism curves of **2** and 10-β-methylselina-4 (14), 7 (11)-diene. Supported, in part by Public Health Service Research Grant No. CA-10737 from the National Cancer Institute

REFERENCES

1. Bohlman, F. and Zeisbert, R. (1975) *Org. Mag. Reson.* **7**, 426.
2. Eggert, H., Van Antwerp, C. L., Bhacca, N. S. and Djerassi, C. (1976) *J. Org. Chem.* **41**, 71.
3. Dalling, D. K., Grant, D. M. and Paul, E. O. (1973) *J. Am. Chem. Soc.* **95**, 3718.
4. Gerber, N. N. (1968) *Tetrahedron Letters* 2971.
5. Senda, Y., Ishiyama, J. and Imaijumi, S. (1975) *Tetrahedron* 1601.
6. Gerber, N. N. (1972) *Phytochemistry* **11**, 385.
7. Maurer, B., Fracheboud, M., Grieder, A. and Ohloff, G. (1972) *Helv. Chim. Acta* **55**, 2371.
8. Wenhert, E., Buckwalter, B. L., Burfitt, I. R., Gasic, M. J., Gottlieb, H. E., Hagaman, E. W., Schell, F. M. and Wookulich, P. M. (1976) in *Topics in Carbon-13 NMF Spectroscopy* (Levy, G. C. ed.). Wiley-Interscience, New York.
9. Ayer, W. A. and Paice, M. G. (1976) *Can. J. Chem* **54**, 910.