SHORT COMMUNICATION

SESQUITERPENOIDS FROM ACTINOMYCETES

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Abstract—A new sesquiterpenol, m p 91°, produced by *Streptomyces fraduae* IMRU 3535 has been shown to be selina-4(14),7(11)-diene-9-ol (I) by its spectral properties and chemical transformations.

INTRODUCTION

RECENTLY we have isolated terpenoids from cultures of actinomycetes. Geosmin $(trans-1, 10\text{-dimethyl-}trans-9\text{-decalol})^1$ and methylisoborneol $(1,2,7,7\text{-tetramethyl-}2\text{-norbornanol})^2$ have subsequently been shown by others to be responsible for earthy/musty odors in public water supplies ³ Three geosmin producing actinomycetes gave, in addition, cadin-4-ene-1-ol,⁴ an enantiomer of epicubenol from cubeb oil ⁵ Streptomyces fradiae IMRU 3535,⁶ the original producer of neomycin⁷ also furnishes geosmin⁸ and a crystalline, faintly odorous, sesquiterpenol described below.

RESULTS AND DISCUSSION

The new sesquiterpenol (I) was obtained in yields of about 5 mg/l. of whole broth from CH_2Cl_2 extracts of whole broth distillates. After preparative GLC it crystallized spontaneously, m.p. 91–92°. The IR spectrum contained strong O—H and C—C bands. The mass spectrum indicated a molecular formula of $C_{15}H_{24}O$ which was verified by analysis. The UV spectrum showed end absorption only, thus eliminating the possibility of conjugated double bonds.

Selenium dehydrogenation of I yielded vetivazulene (4,8-dimethyl-2-isopropylazulene) whose UV and visible absorption spectra were identical with those published ⁹ The structure was confirmed as $C_{15}H_{18}$ by the mass spectrum Vetivazulene was also obtained from I after overnight treatment with 90% formic acid at 50°. The Se dehydrogenation reaction also yielded eudalene (7-isopropyl-1-methylnaphthalene), in major amounts, which was identified by UV, IR and GLC comparisons with an authentic sample. Since I and several derivatives each showed a sharp singlet methyl band in the NMR near 0.8 δ , the carbon

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- ⁸ N N GERBER and H A LECHEVALIER, Applied Microbiol 13, 935 (1965)
- ⁹ (a) B SUSZ, A ST PFAU and PL A PLATTNER, Helv Chim Acta 20, 469 (1937), (b) M SCHOLZ and W TREIBS, Z Elektrochem 65, 120 (1961)

¹ N N GERBER, Tetrahedron Letters 2971 (1968)

² N N GERBER, J. Antibiotics, Tokyo 22, 508 (1969)

³ A A ROSEN, C I MASHNI and R S SAFFERMAN, Water Treatment and Examination 19, 106 (1970)

skeleton was felt to be of the eudesmane or eremophilane type, vetivazulene being formed by a rearrangement reaction

The NMR of I disclosed $H_2C=C$ which was unchanged by acetylation or CrO_3 oxidation The secondary alcohol function was indicated by the NMR bands for CHOH and the oxidation to a ketone The ketone (II) had a UV maximum at 236 nm, a new singlet NMR band characteristic of O=C-CH=C, not O=C-C=CH, and strong O-H and C=O bands in the IR The mass spectrum verified the second oxygen atom (M = 234, $C_{15}H_{22}O_2$) which must be present in a *t*-alcohol group since no further CHOH bands appeared in the NMR A similar double bond migration and allylic hydroxylation accounts for the major products obtained from the CrO_3 oxidation of cholesterol ¹⁰

The olefin groups in I were investigated with the solvomercuration-demercuration reaction ¹¹ This utilizes mercuric acetate in methanol followed by NaBH₄ in dilute NaOH and results in the addition of the elements of methanol to a double bond After 45 min the major product had a GLC retention time higher than that of I After an overnight reaction, the main GLC peak had a still higher retention time than I This, on a submilligram scale, clearly indicated 2 olefin functions of different reactivity Mass spectra confirmed the two products as $C_{16}H_{28}O_2$ and $C_{17}H_{32}O_3$



The above facts can be explained by structures I, III or IV Therefore the saturated substance $C_{17}H_{32}O_3$ was oxidized with CrO_3 The resulting saturated ketone was exchanged twice with NaOD in D_2O^{12} A mass spectrum of the recovered ketone after each exchange clearly showed that 2 atoms of D had been introduced (M = 284), not 3 or 4, thus formulas III and IV were excluded

Selina-4(14),7(11)-diene has recently been isolated from hops ¹³ The NMR of this hops diene and I were identical except for the CHOH in I The IR spectra were very similar and identical in the bands assigned to $H_2C=C$ and $C=CMe_2$ ¹⁴ The hydroxy group in I is equatorially disposed since the NMR band for CHOH in I and for CHOAc in the acetate derivative has a width at half height of 17 cycles/sec^{15,16} In both, the angular methyl band is a sharp singlet which excludes the possibility of an unresolved mixture of isomers ¹⁶

EXPERIMENTAL¹⁷

Preparation and purification of I Streptomyces fraduae IMRU 3535 was maintained on potato-carrot¹⁸ agar slants Growth from 5-7-day-old slants was used to inoculate the seed flasks Whole broth from seed

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- ¹⁸ T Cross, M P Lechevalier and H Lechevalier, J Gen Microbiol 31, 421 (1963)

flasks was inoculated into 6% aq Pablum (60 g Pablum¹⁹/l of tap water) After 7 days of shaking, the whole broth was distilled until 61 had been collected from the typical 101 batch, adding H₂O to the pot as needed A control run with autoclaved Pablum medium alone gave no peaks in GLC assay Extracts from 2 batches were combined for column chromatography The R_f of I on TLC was 0.2 Essentially pure I was obtained from the second silica column The GLC retention times at 180° were I 16.2, anthracene 17.3, and cadin-4-ene-1ol⁴ 9.9 min The mass spectrum of I showed typical sesquiterpenol peaks M-15 (9%), M-18 (30%), M-29 (42%), M-15 and 18 (30%), M-18 and 43 (42%) but an unusually strong molecular ion at 220 mass units (84%) The NMR in CCl₄ showed a sharp singlet at 0.75 δ (angular methyl), a big singlet at 1.78 δ (Me_2 ----C=--C superimposed on methylene absorption) an irregular 4 part multiplet at 3.08-3.35 δ (CHOH) and 2 broad singlets at 4.5 and 4.7 δ (H₂C=--C) There was no change with D₂O Found C, 81.72, H, 11.00 C₁₅H₂₄--O required C, 81.76, H, 10.98 The acetate showed its base peak at 202 mass units (M-HOAc) as well as significant peaks at 187, 159, and 145 mass units The NMR bands in CCl₄ were similar to those of I except for a singlet at 1.95 δ (CH₃CO) and the CHOAc band at 4.3-4.6 δ replaced the CHOH Saponification of the acetate regenerated I

Selenum dehydrogenation of I A dehydrogenation reaction used 50 mg of I, 90 mg of Se and 0.2 ml of linoleic acid as solvent and hydrogen acceptor at 300° for 1 hr Examination of column fractions by TLC disclosed eudalene in earlier fractions as a blue fluorescent spot in UV light, vetuvazulene was evident in later fractions Vetuvazulene, further purified by preparative GLC, crystallized (lit ^{9a}m p 31°) The mass spectrum showed M = 198, M-15 and M-43 The UV spectrum disclosed maxima at 248, 283, 292, 309, 334, 348 (sh), 350, 545, 580 (sh) and 630 (sh) nm Eudalene from I and from a similar Se dehydrogenation of natural eudesmol (eudesma-3 and 4(14)-ene-11-ol) had UV maxima at 264 (sh), 273, 280, 290 (sh), 314, 320 nm, IR maxima at 750, 828, 878, 1373, 1459, 1505, 1607, 1633 cm Retention times at 160°, programmed at 4°/mm were eudalene 10.3, cadalene (1,6-dimethyl-4-isopropylnapthalene) 13.0, vetivazulene 15.3 min, at 180° isothermal anthracene 16, guiazulene (1,4-dimethyl-7-isopropylazulene) 17.3, vetivazulene 19.0 min

Chromic acid oxidation of I Oxidation of 20 mg of I in 1 ml acetone at room temp with 1 ml of Jones reagent (670 mg of CrO_3 in 5 ml of 12% H_2SO_4) for 10 min or with 50 μ l aliquots of Jones reagent added until a yellow color persisted for 30 min gave a mixture of products from which II could be isolated in less than 50% yield The diluted reaction mixture was extracted three times with CHCl₃, the CHCl₃ solution washed, concentrated and applied to a silica column Fractions obtained by eluting with CHCl₃ then EtOAc-CHCl₃ were assayed by GLC In a control reaction I was recovered unchanged from acetone and H_2SO_4 at room temp Oxidation of I (48 mg in 1 ml of pyridine) with Sarett's reagent (100 mg CrO₃ in 1 ml pyridine) overnight at room temp also gave II The retention time of II at 160°, programmed 4°/min was 17 min, at 180° isothermal 24 5 min The mass spectrum showed M at 234 mass units as well as M-15, M-18 and M-33 (=15 + 18) The NMR in CCl₄ showed a singlet at 59 δ (O=C-CH=C), broad singlets OH

at 4 65 and 4 87 δ (H₂C==C) and a big singlet at 1 48 δ (Me₂-C-C=) which in benzene solution was resolved into two CH₃ bands at 1 07 and 1 00 δ Ketone II formed an orange 2,4-dimitrophenylhydrazone m p 195-205°, UV max 380 nm in EtOH

Solvomercuration-demercuration of I A few mg of I was added to a solution of 10 mg HgOAc in 1 ml MeOH The reaction was quenched by the addition of 15 ml of a solution of 15 mg NaBH₄ in 10% NaOH After a few hours to allow the free Hg to coagulate, the mixture was shaken with CH_2Cl_2 and the CH_2Cl_2 solution examined by GLC A 15-min reaction time was sufficient for about half of I to be converted to a product with an 18 min retention time at 160°, 4°/min (the retention time of I under these conditions is 15 min) After preparative GLC it melted at 130–135° and its mass spectrum showed M = 252 as well as M-15, M-18, M-MeOH, MeOH and 18, M-MeOH and 18 and 15 After a 75-min reaction time this product was the major one, 60% or more by peak area analysis A small GLC peak at 21 min retention time was also apparent After an overnight reaction period, the 21 min peak was the main or (70% by peak area analysis) This product was not crystalline after preparative GLC probably because it was a mixture of isomers but its gross structure was verified by mass spectrum. The molecular ion was not visible but its position at 284 was clearly shown byM-CH₃ at 269, M-MeOH at 252, M-MeOH + CH₃ at 237, M-MeOH and H₄O at 234, M-MeOH at 220 mass units

Deuterium exchange of the saturated ketone 50 mg of I was treated, as previously, overnight with 100 mg of HgOAc in 5 ml MeOH The saturated alcohol, after elution from a silica column with CH_2Cl_2 -MeOH, was oxidized with small portions of Jones reagent as already described for I The saturated ketone had a carbonyl band at 5 9 μ in the IR and its mass spectrum showed M at 282 ($C_{17}H_{30}O_3$) with significant peaks at M-Me, M-MeOH, M-MeOH and Me, M-2MeOH The NMR of both saturated alcohol and ketone showed no olefin H bands The saturated ketone (10 mg) was heated at 60° for 2 hr with 1 ml of NaOD in D_2O -dioxane 1 1 prepared by adding 35 mg of clean Na to a mixture of 1 ml D_2O and 1 ml dioxane The reaction mixture was evaporated to dryness under vacuum and the residue taken up in CH₂Cl₂ Preparative

¹⁹ Pablum is a dry, mixed, enriched cereal formerly produced by a division of Mead, Johnson & Co, Evansville, Indiana

GLC furnished a 1 mg portion of saturated ketone for the mass spectrum while the remainder was treated a second time with NaOD in D_2O -dioxane at 60° for 2 hr then overnight at room temp. The relative peak heights at mass numbers 280-285 were before exchange 50, 23, 87, 18, 3, 0, after one exchange 50, 54, 22, 25, 24, 6, after two exchanges 50, 51, 21, 27, 29, 7

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