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VOLATILE LACTONES FROM STREPTOMYCES

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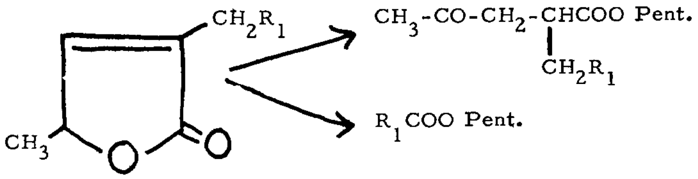
Streptomyces griseus 100-2 (1) was grown in submerged culture on a soybean meal-peptone-salts-glucose medium for 6 days at 28°. Gas chromatography of CH_2Cl_2 extracts of the steam distillate (2) revealed 6 closely and irregularly spaced peaks which were labeled A-F in order of increasing retention time on the non-polar GC column. The relative amounts as indicated by peak areas were D > A > B > C > F > E. The same pattern of peaks at the same retention times but with different relative areas (B > F > C > D > A= E) was obtained from similar extracts of S. odorifer IMRU 3334 but not from the S. odorifer of Collins (3). Identity was established by a comparison of the infra-red spectra of components B and F isolated from both sources and purified by GC.

After preliminary purification on a silica gel column which gave only slight separation of the components (elution order· E, D and C, B, A, E) about 0.5 mg of each was obtained by preparative GC (4). The infra-red spectra of A-E were almost identical with no O-H band, strong C=O at 5.8 μ and 8 other strong bands between 6.9 and 10.6 μ. Components A-E had strong end absorption in the UV and all showed a base peak at 112 mass units. The molecular ions (for A and D 17 and 13%, for B, C and E 3-7% of the base peak) of 154, 168, 182, 182, and 196 combined with the analysis of A (Found C, 72.36, H, 10.45. Calcd. for $C_{10}H_{18}O$, $C_9H_{14}O_2$ or $C_8H_{10}O_3$· C, 79.0, 72.5 or 66.9, H, 12.1, 9.9 or 7.7) established the molecular formulas as $C_9H_{14}O_2$, $C_{10}H_{16}O_2$, $C_{11}H_{18}O_2$, $C_{11}H_{18}O_2$ and $C_{12}H_{20}O_2$. The log of the retention times versus number of carbons showed that A and D belonged to one homologous series and B, C, and E to another. The $[\alpha]_D^{25}$ of D was +44°, at 578, 546, 436 and 365 nm the $[\alpha]$'s were +45°, +51°, +93° and +154°.

The nmr of A disclosed peaks at 6.8δ (1 H, narrow multiplet,), 4.9δ (1 H, broad multiplet), 2.2δ (2 H, broad multiplet), 1.4δ (3 H, doublet J= 7), 1.06 δ (3 H, narrow multiplet). Later the nmr of B was found to be similar with bands at 6.9δ (1 H, narrow multiplet), 4.9δ (1 H, broad multiplet), 2.25δ (2 H, broad multiplet), 1.4δ (3 H, doublet, J= 6), 0.95δ (6 H, J= 6). For α, β unsaturated γ-lactones a chemical shift of 6.8-6.9δ is characteristic of hydrogen at the β position, not α (5). In many examples -C$\underline{H}$-O is between 4.8 and 5.1δ and the coupling constants with the olefinic hydrogen are small resulting in a relatively narrow band at 6.9δ . A γ-lactone structure with an exocyclic, conjugated double bond is excluded since it would have 4 H at 2.2-2.5δ ($C\underline{H}_2$-C= C-) not 2, -C$\underline{H}$-O at 4.3δ (9), and the band at 6.9δ would be broad due to the normal J vicinal of 4-10 cps for freely rotating systems. Typical saponification reactions of A-E failed to furnish volatile acids (identified by odor) or recognizable alcohols (identified by GC). From refluxing EtOH-HCl A-E were recovered unchanged, a procedure known to convert β, γ unsaturated-γ-lactones to the keto esters (6).



A. R_1 = $-CH_2CH_2CH_3$.

B. R_1 = $-CH_2CH(CH_3)_2$.

C. R_1 = $-CH_2CH_2CH(CH_3)_2$.

D. R_1 = $-(CH_2)_4CH_3$.

E. R_1 = $-(CH_2)_3CH)CH_3)_2$.

These structures were confirmed by chemical degradations. Crude A-F was kept over night at 50° in EtOH-dilute NaOH 1 2. After 5 fold dilution and acidification the residue from the CH_2Cl_2 extraction was esterified by standing overnight at 50° with 0.5 ml benzene, 0.1 ml pentanol, 0.025 ml conc. H_2SO_4 and a piece of Drierite. The benzene supernatant was shaken with aqueous bicarbonate, clarified by standing with Drierite and GC'd directly. A pattern of peaks (A_1-E_1) similar to A-E was observed at a significantly higher retention time. Transesterification with heptyl alcohol showed that A_1-E_1 were esters. In a control reaction GC purified D furnished D_1 which gave a positive microtest for carbonyl with 2,4-dinitrophenylhydrazine and whose IR showed C=O but no O-H. Mass spectra of A_1 and B_1 showed molecular ions (2%) corresponding to $C_{14}H_{26}O_3$ and $C_{15}H_{28}O_3$, thus all the carbon atoms in the original lactone were accounted for.

Crude A-F after overnight at 50° in 1% NaOH was diluted 5 fold and treated with concentrated aqueous $KmnO_4$ until a pink color persisted for 2-3 hours. After centrifugation to remove MnO_2, decolorization with EtOH and recentrifugation, the solution was acidified, extracted and esterified as above. GC revealed a pattern of peaks similar to A-E at a significantly lower retention time (A_2-E_2) except that the A_2 peak was unusually large. A control esterification showed that some dipentyl ether was formed which cochromatographed with A_2. The retention times of A_2, B_2, C_2 and D_2 were identical with those of authentic samples of the n-pentyl esters of n-butyric, isovaleric, isocaproic and n-caproic acids. In a control reaction GC purified C and D furnished C_2 and D_2. The IR of D_2 showed a strong C=O band. The mass spectra of C_2 and D_2 verified them as isomeric pentyl hexanoates since strong peaks were observed for both at 117 $(RCOO + 2H)^+$ and 99 $(RCO)^+$ mass units (7). Clearly A_2-E_2 were derived from the variable portions of A-E. Apparently the α-keto acids formed initially by oxidative cleavage of the double bonds were sufficiently enolized in the alkaline solution for further oxidation to occur. In a control reaction α-hydroxylauric acid and dilute alkaline permanganate furnished decanoic acid.

Footnotes and References

1. This culture was received from Miss Lilia McMillan of the Central Water Filtration Plant, Chicago, Ill. and identified by Mrs. M. P. Lechevalier of this Institute. It had been isolated from Lake Michigan water.

2. The experimental procedures were similar to those described in N. N. Gerber and H. A. Lechevalier, *Appl. Microbiol.* **13**, 935 (1965).

3. R. P. Collins, L. E. Knaak and J. W. Soboslai, *Lloydia* **33**, 199 (1970).

4. The odor of geosmin coincided with the leading edge of peak C. Some of the oil collected at this time was saponified with 20% EtOH-KOH overnight at 60°. The CH_2Cl_2 extract of the alkaline reaction mixture gave only one GC peak, identical in odor and retention time with authentic geosmin (N. N. Gerber, *Tet. Letters* 2971 (1968). Geosmin probably also co-chromatographed with a $C_{11}H_{16}O_2$ lactone see The structure of mucidone, G. Sipma, B. van der Wal and D. K. Kettenes, *Tet. Letters* 4159 (1972).

5. For nmr of similar lactones see S. Ducher and A. Michet, *Compt. Rend. Acad. Sci. Paris* **267**, 1617 (1968) (by error the values for the olefinic protons in 2-pentene-4-olide were exchanged, S. Ducher, private communication), A. Loeffler, F. Norris, W. Taub, K. L. Svanholt and A. S. Dreiding, *Helv. Chim. Acta* **53**, 403 (1970), L. Avellen, I. Crossland and K. Lund, *Acta Chim. Scand.* **21**, 2104 (1967), M. Klaren-deWit, D. J. Frost and J. P. Ward, *Rec. Trav. Chim. Pays-Bas* **90**, 1207 (1971), P. E. Sonnet, *J. Org. Chem.* **33**, 3662 (1968), T. C. McMorris, *ibid* **35**, 458 (1970), L. M. Jackman and S. Sternhell, Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, 2nd Ed., Pergamon Press 1969. p. 188.

6. F. A. Kuehl, R. P. Linstead and B. A. Orkin, *J. Chem. Soc.* 2213 (1950).

7. J. H. Beynon, R. A. Saunders and A. E. Williams, *The Mass Spectra of Organic Molecules*, Elsevier, 1968, p. 241.

8. We are indebted to Dr. Sipma and Dr. D. K. Kettenes of Polak's Fruital Works, N. V., Amersfoort, Holland for the nmr of B and for valuable suggestions. We thank Dr Dorothy Denny for nmr spectra, Mrs. E. M. Fekete for technical assistance and the U. S. Public Health Service AI-06708-05 for partial financial support.

9. L. M. Jackman and S. Sternhell, ref. (5) p. 198.