Tetrahedron Letters No.5, pp. 381-382, 1970. Pergamon Press. Printed in Great Britain.

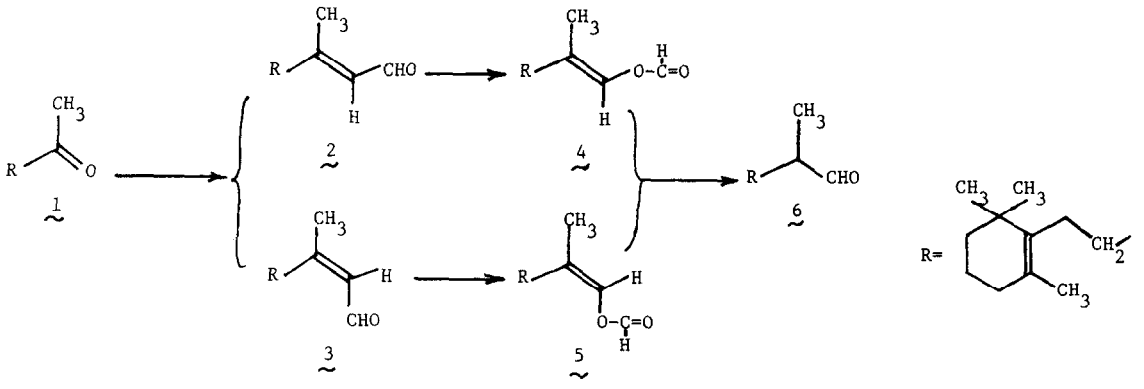
SYNTHESIS AND STEREOCHEMISTRY OF LATIA LUCIFERIN

Fumiaki Nakatsubo, Yoshito Kishi and Toshio Goto
Department of Agricultural Chemistry, Nagoya University, Chikusa, Nagoya, Japan

(Received in Japan 29 November 1969; received in UK for publication 29 December 1969)

The novel enol formate structure 4[1] (except stereochemistry) has been proposed for Latia luciferin, the specific substrate in bioluminescence enzyme (luciferase) system in the fresh water limpet Latia neritoides. The proposed structure has been confirmed recently by synthesis[2]: the key step in the synthesis was acylation of desformyl luciferin 6[1] by formic acetic anhydride. However, the yield of the key step is low (3%) and the geometrical configuration associated with the double bond in the side chain is left to be solved. We would like to report the alternative synthesis of the luciferin through Baeyer-Villiger rearrangement of α,β-unsaturated aldehyde 2 and to discuss the stereochemistry of the luciferin.

Dihydro-β-ionone 1[3] was added dropwise at -70° into lithium ethylidenecyclohexylamide[4] prepared from ethylidenecyclohexylamine and lithium diisopropylamide in ether at 0°, being stirred at r.t. for 40 hr. Hydrolysis of the resultant aldimine adduct with acetic acid gave a mixture[5] of trans- and cis-α,β-unsaturated aldehyde in 90% yield. Thick layer chromatography of the mixture on silica gel with n-hexane-ether(10:1) gave pure trans-aldehyde 2 (40%) as a viscous oil [nmr($CDCl_3$): 1.02 (s, 6H), 1.63(broad s, 3H), 2.23(d, J=1.2 Hz, 3H), 5.93(broad d, J=8 Hz, 1H), 10.00(d, J=8 Hz, 1H); ir(CCl_4): 1678 cm^{-1}; uv(CH_3OH): 237 mμ(ε=13,800); ms(heating, 70eV): 220, 205, 176, 161, 137, 121; vpc[6](120°): 9.5 min] and pure cis-aldehyde 3 (17%) as a viscous oil [nmr($CDCl_3$): 1.03(s, 6H), 1.67 (broad s, 3H), 2.04(d, J=1.2 Hz, 3H), 5.89(broad d, J=8 Hz, 1H), 10.00(d, J=8 Hz, 1H); ir(CCl_4): 1678 cm^{-1}; uv(CH_3OH): 237mμ(ε=12,300); ms(heating, 70eV): 220, 205, 176, 161, 137, 121; vpc(120°): 7.6 min].



The configuration of aldehyde 2 and 3 was determined from nmr spectra. Tori et al.[7] examined carefully the nmr spectra of trans- and cis-citral and concluded that the methyl signal on α,β-unsaturated aldehyde moiety of the trans-isomer (formyl group is cis to methyl group) appears in lower field than that of cis-citral because of the difference in magnetic shielding effects of

formyl groups. As the situation of the methyl group in 2 and 3 is exactly same as that in citral, it can be concluded that the double bond in the major product has trans-orientation (formyl group is cis to methyl group) and the minor product cis-orientation.

Treatment of trans-aldehyde 2 with anhydrous hydrogen peroxide in t-amyl alcohol in the presence of selenium dioxide[8] at 70° for 2 hr gave trans-enol formate 4 in high yield.[9] The product [50%; nmr(CCl_4): 1.00(s, 6H), 1.60(broad s, 3H), 1.72(d, J=1.3 Hz, 3H), 6.97(broad s, 1H), 7.95(s, 1H); ir(CCl_4, 0.5 mm): 1738, 1162 cm^{-1}; uv(EtOH, end absorption): ε=7,000 at 220 mμ, 8,100 at 210; ms(heating, 70eV): 236, 190, 175, 137; vpc(110°): 5.8 min] was easily isolated by thick layer chromatography on silica gel with n-hexane-ether(20:1) and found to be identical with natural Latia luciferin in every respect.[10] From cis aldehyde 3 cis-enol formate 5, isomeric to 4, was formed in high yield[9] under the same condition used in the case of 2. The isolated product [55%; nmr(CCl_4): 0.99(s, 6H), 1.60(broad s, 3H), 1.68(d, J=1.3 Hz, 3H), 6.89(broad s, 1H), 7.93(s, 1H); ir(CCl_4, 0.5 mm): 1738, 1165 cm^{-1}; uv(EtOH, end absorption): ε=8,000 at 220 mμ, 8,900 at 210; ms(heating, 70eV); 236, (208), 190, 175, 137; vpc(110°): 4.9 min] is difinitely different from the trans-isomer 4, although the spectroscopic data of 5 are quite similar to those of 4. The criteria to distinguish 5 from 4 are obtained from the nmr spectra (the differences of the chemical shift of olefinic proton and the shape of signals around 1.8-2.2 ppm) and from the retention time of vpc, so far. Attempted purification of 4 and 5 with aluminum oxide plates gave desformyl luciferin 6[11] in almost quantitative yield. The synthetic luciferin had 80-100% activity, but cis-isomer 5 had 50-60% activity of the natural luciferin against Latia luciferase in the preliminary experiments.[12]

From the fact that Bayer-Villiger rearrangement proceeds under complete retention of configuration on a migrating group[13] and the fact that only trans-aldehyde 2 gives Latia luciferin, the geometrical configuration associated with the double bond in the natural luciferin must have trans-orientation (O=C(H)-O-group is cis to CH_3-group).

REFERENCES AND FOOTNOTES

1. O. Shimomura and F.H. Johnson, Biochemistry 7, 1734 (1968)
2. M.G. Fracheboud, O. Shimomura, R.K. Hill, and F.H. Johnson, Tetrahedron Letters 3951 (1969)
3. A. Caliezi and H. Shierg, Helv. Chim. Acta. 33, 1129 (1950)
4. G. Wittig and H. Reiff, Angew. Chem. intern. Edit. 7, 7 (1968)
5. The mixture contained 69% of trans- and 31% of cis-aldehyde from vpc analysis.
6. Vpc condition used in this work: DEGS 12% on Anakrom A (60-70 mesh), 3 mm-2 m column.
7. M. Ohtsuru, M. Teraoka, K. Tori, and K. Takeda, J. Chem. Soc. (B) 1033 (1967)
8. C.W. Smith and R.T. Holm, J. Org. Chem. 22, 746 (1957)
9. No by-product was detected by vpc analysis.
10. For the spectroscopic data of natural luciferin, see reference 1.
11. Nmr(CCl_4): 0.99(s, 6H), 1.11(d, J=7 Hz, 3H), 1.58(broad s, 3H), 9.58(d, J=1.5 Hz, 1H); ir(CCl_4): 1728 cm^{-1}; ms(heating, 70eV): 208, 193, 175, 150, 135, 123.
12. The authors are indebted to Dr. O. Shimomura, Princeton University, for his testing the bioluminescence activity of 4 and 5 and for his sending the copies of nmr and ir spectra of natural Latia luciferin.
13. P.A.S. Smith, "Rearrangements Involving Migration to an Electron-Deficient Nitrogen or Oxygen", in P. de Mayo, Molecular Rearrangements, Vol. 1, p. 577, Interscience Publishers, New York (1963)