

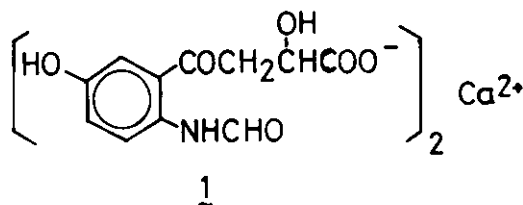
BIOGENETIC TYPE SYNTHESIS OF BLEPHARISMONE,<sup>1</sup> A CONJUGATION  
INDUCING GAMONE IN CILIATE BLEPHARISMA

Takashi Tokoroyama,\* Yoshinori Kawasaki, Miwako Nakatani  
and Kozo Shibata

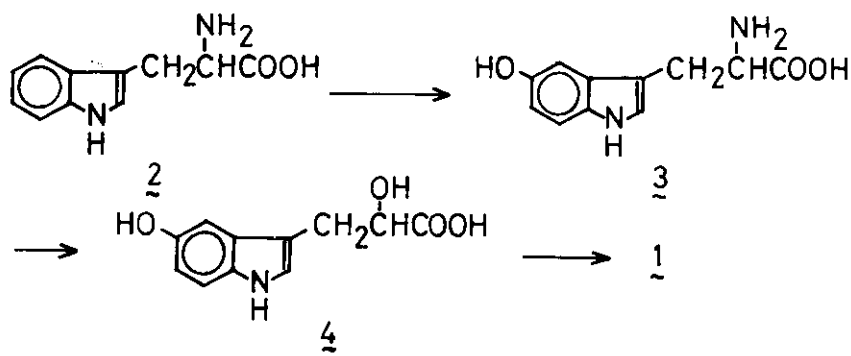
Faculty of Science, Osaka City University, Sumiyoshi-ku  
Osaka 558, Japan

Blepharismone 1 was synthesized by oxidation of benzyl 5-benzyloxyindolylactate 5 followed by de-protection, which was prepared by the reaction of 5-benzyloxyindolylmagnesium iodide 9 with benzyl glycidate 7.

Blepharismone 1 is one of two gamones which are excreted respectively by the complementary mating types of a ciliate, Blepharisma intermedium and respectively induce conjugation of the other type of cells.<sup>4,5</sup> It has been isolated in a crystalline form and found to have the structure of calcium 3-(2'-formylamino-5'-hydroxybenzoyl)-lactate 1.<sup>2,6</sup> In view of the unique biological activity



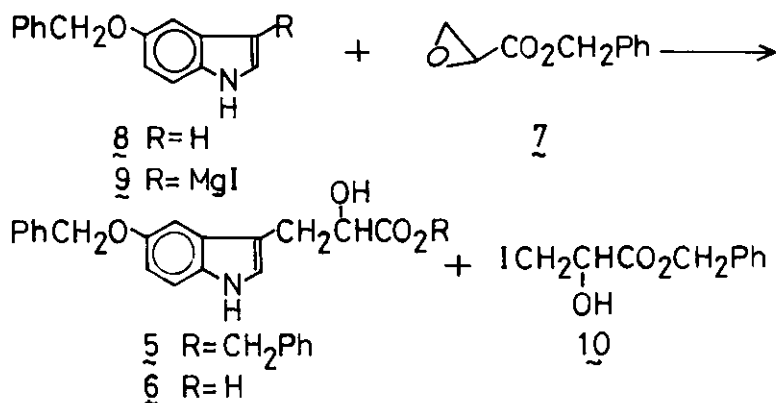
of blepharismone 1, the establishment of the efficient method preparing it by chemical synthesis would be mandatory for the molecular-biological studies of conjugation mechanism in the Protozoa. Previously we reported on the synthesis of 1 by the aldol condensation of 2-formylamino-5-hydroxyacetophenone with sodium glyoxylate but this method was far from practical since isolation procedure was tedious (repeated preparative TLC) and the yield was extremely low.<sup>8</sup> In the continuation of our work to find more satisfactory methods we have investigated the synthesis of 1 from indole precursor by the cleavage of  $\Delta^{2,3}$ -double bond, which represents the supposed biosynthetic process (cf. Scheme 1).<sup>9</sup>



Scheme 1

We planned to synthesize an appropriately protected derivative of 5-hydroxyindolylactic acid 4 first and then to convert it to 1 by oxidative cleavage followed by deprotection and salt formation. Since blepharismone 1 is sensitive to both acidic and basic condition, benzylic group was selected as the protecting group and thus we firstly concerned with the synthesis of the key

intermediate 5. The synthesis of the acid 6 corresponding to 5, based on the reaction of 5-benzyloxygramine with diethyl acetoxy-malonate, has been reported.<sup>10</sup> In attempt to prepare 5 more efficiently, we studied the reaction of benzyl glycidate 7<sup>11</sup> with 5-benzyloxyindolylmagnesium iodide 9. The Grignard reaction of glycidic esters is expected to yield the product substituted at  $\beta$ -carbon atom,<sup>13,14</sup> as is the case in the reactions with most of nucleophiles.<sup>15,16</sup> A solution of 9 in ether-benzene (1:1) prepared from the reaction of 8 with methyl magnesium iodide was treated with an ether solution of 7 (2 equiv.) at 0° overnight and, after chromatographic separation (SiO<sub>2</sub>) and crystallization, two products, 5, m.p.90° (13.5% yield) and benzyl 3-iodo-2-hydroxypropionate 10, m.p.66° (38% yield) were identified with some recovery of 8 (26.5%). That the former compound represented the desired product, was indicated in its NMR spectrum (CDCl<sub>3</sub>) ( $\delta$ ): 2.82(1H, d, J=6 Hz, -OH), 3.16(2H, m, -CH<sub>2</sub>CH(OH)CO<sub>2</sub>-), 4.45(1H, dt, J=5 and 6 Hz, -CH<sub>2</sub>CH(OH)CO<sub>2</sub>-), 4.98, 5.03(each 2H, s, -CH<sub>2</sub>Ph), 6.70-7.40(13H, m, ArH), 7.90(1H, br s, -NH-). Upon addition of a



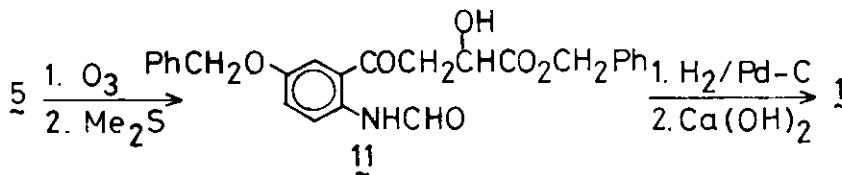
Scheme 2

drop of trifluoroacetic acid, the signals at  $\delta$  2.82 and 7.90 had disappeared and the doublet of triplets at  $\delta$  4.45 changed to a triplet. The formation of 10 is interpreted as the result of the reaction of 7 with magnesium iodide present in an equilibrium mixture of the Grignard reagent.

Next phase of the synthesis was the oxidative cleavage of the indole ring at  $\Delta^{2,3}$ -double bond in 5. Various chemical methods to mimitate this significant biosynthetic reaction have been reported.<sup>17</sup> Of these we tested sodium periodate oxidation and ozonolysis, the latter being found to be more satisfactory. 5 in ethyl acetate solution was carefully ozonized at  $-70^\circ$  in such way as the bubbling of ozone stream was stopped immediately after the firstly developed yellow tint of the solution had turned to pale blue by the excess of ozone. The ozonized solution was treated with dimethyl sulfide and the product was isolated to give 11, m.p.  $108^\circ$ , IR(CHCl<sub>3</sub>): 3510, 3260, 1730, 1690, 1665 cm<sup>-1</sup>; NMR(CDCl<sub>3</sub>) ( $\delta$ ): 3.45(1H, d, J = 6Hz, -OH), 3.51(2H, distorted d, J=6 Hz, -COCH<sub>2</sub>CH(OH)CO<sub>2</sub>-), 4.58 (1H, q, J=6 Hz, -COCH<sub>2</sub>CH(OH)CO<sub>2</sub>-), 5.09, 5.26(each 2H, s, -CH<sub>2</sub>Ph), 7.1-7.6(13H, m, ArH), 8.37, 8.39(1H in total, each s, -NHCHO),<sup>18</sup> 8.65, 8.74(1H in total, each s, -NHCHO)<sup>18</sup> in 49% yield.

Finally 11 was debenzylated by hydrogenation (10% Pd-C in ethanol). The product<sup>20</sup> was dissolved in water and a saturated solution of calcium hydroxide was added. The crystals, which deposited on standing, were collected and purified by recrystallization from water to yield 1 as pale yellow crystals. The identity of this substance with natural blepharismone was confirmed by the comparison of IR(KBr),<sup>21</sup> NMR(D<sub>2</sub>O) and TLC with three different

solvent systems [Merck, Pre-coated TLC plates, cellulose; propanol-H<sub>2</sub>O(3:1); butanol-acetic acid-H<sub>2</sub>O(12:3:5); t-butyl alcohol-conc. NH<sub>3</sub>-H<sub>2</sub>O(3:1:1)].

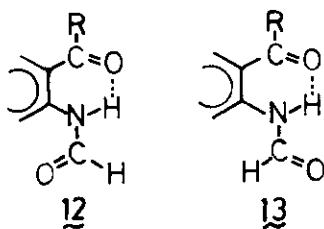


Scheme 3

## REFERENCES

1. This compound was firstly named as blepharismine<sup>2</sup> and then renamed.<sup>3</sup>
2. T. Kubota, T. Tokoroyama, T. Tsukuda, H. Koyama and A. Miyake, Science, 179, 400(1973).
3. Cf. note 3 of ref. 7.
4. A. Miyake, Proc. Japan Acad., 44, 837(1968).
5. A. Miyake and J. Beyer, Exp. Cell Res., 76, 15(1973).
6. The other gamone, blepharmone was also isolated and identified as a glycoprotein.<sup>7</sup>
7. A. Miyake and J. Beyer, Science, 185, 623(1974).
8. T. Tokoroyama, S. Horii and T. Kubota, Proc. Japan Acad., 49, 461(1973).
9. The sequence of the steps involved in the scheme has not been established yet.
10. M. J. Gortatowski and M. D. Armstrong, J. Org. Chem., 22, 1217 (1957).

11. 7 was prepared by the treatment of potassium glycidate<sup>12</sup> with benzyl bromide in tetrahydrofuran solution.
12. N. F. Blau, J. W. Johnson and C. G. Stuckwisch, J. Am. Chem. Soc., 76, 5106(1954).
13. The reaction of indolylmagnesium halide with ethyl 2,3-epoxybutyrate is reported to give  $\beta$ -cleavage product without experimental details. M. S. von Wittenau and H. Els, J. Am. Chem. Soc., 85, 3425(1963).
14. Rather complicated results were also reported. N. G. Gaylord and E. I. Becker, Chem. Rev., 49, 413(1951).
15. R. E. Parker and N. S. Isaacs, Chem. Rev., 59, 737(1959).
16. J. G. Buchanan and H. Z. Sable, 'Selective Organic Transformations', eds. B. S. Thyagarajan, Vol. 2, Wiley-Interscience, New York, 1972, p. 8.
17. Peracid oxidation: W. E. Savige, Aust. J. Chem., 28, 2275 (1975); periodate oxidation: A. J. Fatiadi, Synthesis, 239 (1974); metal-catalyzed oxygenation: K. Uchida, M. Soma, S. Naito, T. Onishi and K. Tamaru, Chem. Lett., 471(1978); photooxygenation: I. Saito, M. Imuta, Y. Takahashi, S. Matsugo and T. Matsuura, J. Am. Chem. Soc., 99, 2006(1977); M. Nakagawa, H. Okajima and T. Hino, J. Am. Chem. Soc., 99, 4424(1977); ozonolysis: F. Sakiyama, N. Masuda and T. Nakagawa, Chem. Lett., 893(1978) and references cited therein.
18. The presence of these signals in pairs indicate that in the solution 11 exists as a mixture of two conformers 12 ( $\delta$  8.39 and 8.65) and 13 ( $\delta$  8.37 and 8.74).<sup>19</sup> The inspection of integral showed 12 predominated slightly. At 50° the pairs



of signals collapsed respectively to broad singlets and at the same time the signal due to -NH- group naturally shifted to higher field.

19. J. W. Emsley, J. Feeney and L. H. Sutcliffe, 'High Resolution Nuclear Magnetic Resonance Spectroscopy', Vol. 1, Pergamon, Oxford, 1965, p. 553.
20. The attempts to characterize the free acid corresponding to 1 were not successful.
21. The IR spectra of synthetic and natural blepharismone 1 exhibited slight differences in the region near 1400 and 1250  $\text{cm}^{-1}$  since both spectra concerned with those of solid state (racemic and optically active crystals respectively).

Received, 4th October, 1978