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Synthesis of hipposudoric and norhipposudoric acids: the pigments responsible for the color reaction of the red sweat of *Hippopotamus amphibius*

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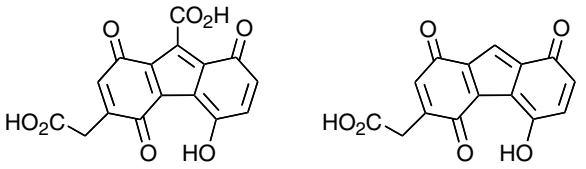
Abstract—Highly unstable pigments, hipposudoric acid and norhipposudoric acid, isolated from the red sweat of hippopotamus were synthesized using the Pschorr reaction for the construction of the fluorene nucleus as the key step and the careful oxidation in the last step.

Hippopotamuses secrete a viscous alkaline liquid over their face and back. The colorless sweat (secretion) turns red within a few minutes, then gradually becomes brown polymers. The colored sweat has been said to protect the hippopotamus' skin from sunlight and from infection by microbes.[1] We focused on this color reaction of the sweat, and recently isolated the red and orange pigments responsible for the color reaction, determined their structures, and partly clarified their functions.[2] We designated the red and orange pigments, hipposudoric acid (**1**) and norhipposudoric acid (**2**), respectively (Fig. 1). In order to investigate in more detail the chemical and biological properties of these scarcely available and ephemeral compounds, we decided to synthesize these pigments. The synthetic strategy is outlined in Scheme 1. The easily polymerizable target compounds **1** and **2** would be obtained by oxidation of the corresponding hydroquinones **3** and **4** with spontaneous enolization.[2] Each carboxylic acid would be available by the one-carbon elongation (for **4**) or double one-carbon elongation



Hipposudoric acid (1) **Norhipposudoric acid (2)**

Figure 1.

(for **3**) of the key intermediate **5**. Furthermore, the fluorenone skeleton of **5** would be constructed from **6** by the Pschorr reaction.[3] Thus the precursor of **6** was analyzed into **7** and **8**.

The commercially available 2,5-dimethoxytoluene (**7**) was brominated with Br_2 in CH_2Cl_2 to afford the desired substitution product, 4-bromo-2,5-dimethoxytoluene (**9**)[4] (Scheme 2). The other component, 3,6-dimethoxy-2-nitrobenzaldehyde (**10**), was synthesized from the commercially available 2,5-dimethoxybenzaldehyde (**8**) by nitration with nitric acid,[5] giving a 3:1 mixture of the *o*- and *p*-nitro products, **10** and **11**, respectively;[6] from which the desired **10** was obtained by recrystallization. The coupling reaction of the aryllithium derived from bromide **9** and *t*-BuLi with aldehyde **10** in THF[7] followed by oxidation using PCC in CH_2Cl_2 afforded ketone **12** in excellent yield (96% for two steps). The nitro group of **12** was reduced with iron in aqueous AcOH to give amine **6** in 86% yield,[8] which was oxidized to the

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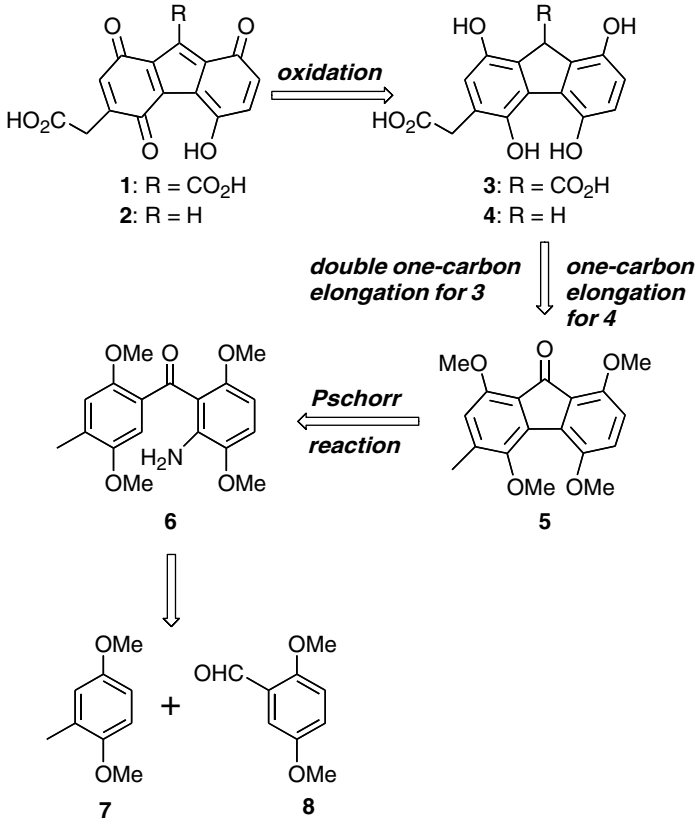
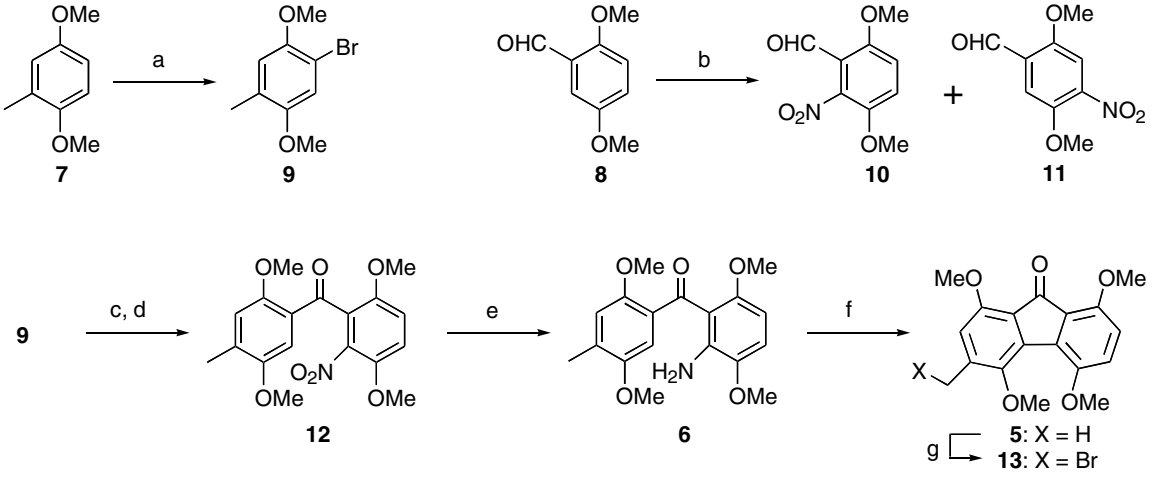


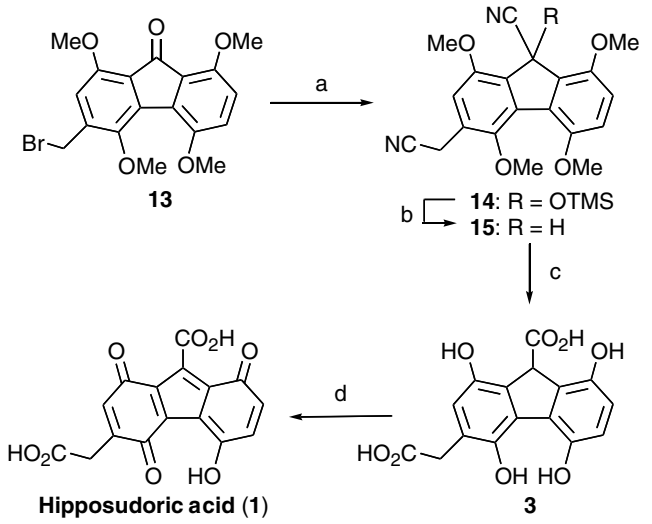
Scheme 1. Retrosynthesis of hipposudoric acid (**1**) and norhipposudoric acid (**2**).



Scheme 2. Synthesis of the key intermediate **13**. Reagents and conditions: (a) Br_2 (1.05 mol amt), CH_2Cl_2, rt, 10 h, 75%; (b) concd HNO_3, 0 °C, 5 min, 57% of **10** (after recrystallization from hexane–$CHCl_3$); (c) **9** (1.0 mol amt), *t*-BuLi (1.5 mol amt)/pentane, THF, −78 °C, 15 min, then **10** (0.8 mol amt), −78 °C to rt, 2 h; (d) PCC (1.6 mol amt), NaOAc, CH_2Cl_2, rt, 12 h, 96% (two steps); (e) Fe (3.6 mol amt), AcOH–H_2O (9:1), 80 °C, 1 h, 86%; (f) isoamyl nitrite (2.0 mol amt), AcOH, rt, 0.5 h, then hydroquinone (1.2 mol amt)/acetone, rt, 1 h, 79%; (g) NBS (1.0 mol amt), BPO (0.06 mol amt), benzene, reflux, 13 h, 92%. PCC = pyridinium chlorochromate, NBS = *N*-bromosuccinimide, BPO = benzoyl peroxide.

diazonium salt and then smoothly converted to the desired fluorenone **5** (79%) by the modified Pschorr ring-closing reaction promoted by hydroquinone.[9] Benzylic bromination of **5** with NBS in the presence of a catalytic amount of BPO in benzene gave **13** in 92% yield as the key intermediate for the synthesis of **1** and **2**.

For the synthesis of hipposudoric acid (**1**), the double one-carbon elongation on **13** was realized. The simultaneous substitution and addition of the cyano groups using TMSCN afforded the cyanohydrin TMS ether **14** in 69% yield (Scheme 3).[10,11] In this reaction, the cyanide ion released from TMSCN and TBAF is used for the

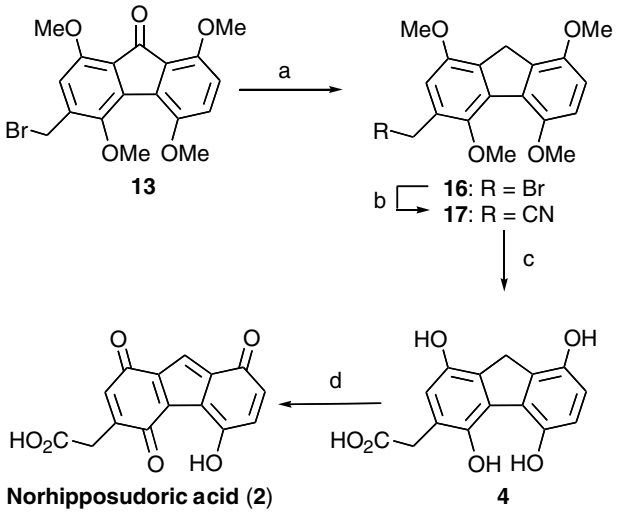


Scheme 3. Synthesis of hipposudoric acid (**1**). Reagents and conditions: (a) 1 M TBAF in THF (1.6 mol amt), TMSCN (solvent, 18 mol amt), rt, 15 min, 69%; (b) Et_3SiH (5.0 mol amt), $BF_3{\cdot}Et_2O$ (5.0 mol amt), CH_2Cl_2, 0 °C, 1 h, 99%; (c) 48% aq HBr–AcOH (3:1), reflux, 4 h; (d) $CuSO_4$ (1.0 mol amt), 0.5 M aq $NaHCO_3$, rt, 2 min, 35% (two steps). TBAF = tetrabutylammonium fluoride, TMS = trimethylsilyl.

substitution reaction[10] and also catalyzes the addition of TMSCN to the ketone.[11] The resulting cyanohydrin **14** was deoxygenated with Et_3SiH and $BF_3{\cdot}Et_2O$ to quantitatively give nitrile **15**.[12] Having the desired compound with the appropriate skeleton, the synthesis reached its final stage. Both hydrolysis of the two nitrile groups and cleavage of the four methyl ethers were achieved using aqueous HBr–AcOH to afford the highly unstable hydroquinone **3**. Even if ^1H NMR analysis of the crude product showed the quantitative formation of **3**, purification by SiO_2 or ODS column chromatography resulted in the decomposition of the product because of its instability in air. Therefore, the crude product was directly subjected to the last step. Oxidation of the two hydroquinone groups under the usual conditions such as $FeCl_3$–HCl[13] and Ag_2O[14] resulted in failure, giving brown polymers. In some cases ($FeCl_3$–MeOH, $FeCl_3$–MeCN), the desired oxidation should have proceeded judging from the appearance of the red coloration of the solution; however, the subsequent polymerization was too fast to isolate the red compound. Simple dilute conditions had no effect on retarding the polymerization. After considerable efforts, we succeeded in this oxidation using $FeCl_3$ in a viscous solvent, glycerol–H_2O (10:1), in order to prevent the substrates from approaching each other. To obtain a sufficient amount of **1**, careful treatment during the purification step was required. The resulting reaction mixture was immediately filtered through a cation exchange resin (CM Sephadex, 0.2 M phosphate buffer, pH 6.1) to remove the iron ion. The obtained red solution was subjected to anion exchange chromatography similar to the purification of the natural product (QAE Sephadex, 1.7 M NaCl in 0.2 M phosphate buffer, pH 6.1)[2] to afford highly unstable hipposudoric acid (**1**) as a dilute buffer solution in 10% yield (from **15**) judging from the ^1H NMR and UV–vis spectra.[15] We examined the milder and more efficient oxidation methods and found that the Cu^{2+}-catalyzed air oxidation[16] was the more appropriate method because of lower degree of polymerization. Interestingly, the results of this oxidation depended on the pH of the reaction medium. The oxidation of **3** under the basic conditions (0.5 M aqueous $NaHCO_3$, pH 8.3) afforded **1** in 35% yield from **15** along with the negligible yield of **2**.[15] On the other hand, the same oxidation in an acidic buffer solution (0.2 M phosphate buffer, pH 6.1) led to the formation of **1** (8.4%) along with **2** (6.8%).[15] The orange pigment **2** would occur due to the competitive decarboxylative enolization after oxidation.

The other target molecule, norhipposudoric acid (**2**) (the orange pigment), was also synthesized from the intermediate **13** (Scheme 4). The carbonyl group of **13** was reduced with Et_3SiH in TFA[17] to afford fluorene **16** in 79% yield. Substitution of the bromide with the cyanide was performed by the standard method (NaCN, DMF) to give benzylnitrile **17** in 80% yield. Hydrolysis of the nitrile and demethylation of **17** were conducted using a similar method as that in the case of **15**, giving hydroquinone **4**. This hydroquinone was also unstable; therefore, the crude product was immediately oxidized with $CuSO_4$ in 0.2 M phosphate buffer (pH 6.1) to produce the orange solution. This solution was immediately subjected to filtration through a cation exchange resin (CM Sephadex, 0.2 M phosphate buffer, pH 6.1) followed by anion exchange chromatography (QAE Sephadex, 2.3 M NaCl in 0.2 M phosphate buffer, pH 6.1) to afford norhipposudoric acid (**2**) in 36% yield from **17** as a dilute buffer solution.[15] The analytical data of these synthesized pigments were identical to those of the natural products.

In conclusion, the syntheses of hipposudoric acid (**1**) and norhipposudoric acid (**2**) were performed by featuring the Pschorr ring-closing reaction, cyanation to



Scheme 4. Synthesis of norhipposudoric acid (**2**). Reagents and conditions: (a) Et_3SiH (5.0 mol amt), TFA, 0 °C, 15 min, 79%; (b) NaCN (1.8 mol amt), DMF, 50 °C, 0.5 h, 80%; (c) 48% aq HBr–AcOH (3:1), reflux, 4 h; (d) $CuSO_4$ (1.0 mol amt), 0.2 M phosphate buffer, rt, 2 min, 36% (two steps).

introduce the carboxylic acid function, and the careful oxidation of bis-hydroquinone to bis-quinone. Further chemical and biological studies using these pigments and their analogs are now under way.

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