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# First total synthesis of structurally unique flavonoids and their strong anti-inflammatory effect

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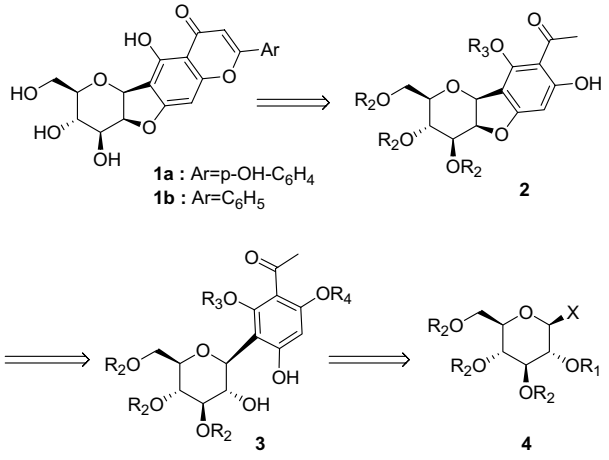
**Abstract**—The first total synthesis of structurally unique flavonoids **1a** and **1b** is described. These compounds showed very strong anti-inflammatory effect against delayed hypersensitivity in a mouse model.


Flavonoids, including apigenin, luteolin, diosmin, and rutin show diverse biological properties[1] such as anti-inflammatory, anti-cancer, and anti-oxidant effects. Recently, Ishikura et al. discovered a novel and structurally unique flavonoid (**1a**) from oolong tea extract, which showed very strong anti-inflammatory activity.[2] Although structurally related flavone C-glycosides such as vitexin and isovitexin are widespread in nature and a synthetic method was also investigated,[3] a fused tricyclic flavone, such as **1**, has not been found.

In our effort to exploit a new anti-inflammatory agent, we are very interested in **1a** and its derivatives, but the content of **1a** in oolong tea is very low. In addition, there are no reports of synthetic methods for this type of compound. Here, we wish to report the first total synthesis of **1a** and its derivative (**1b**) and their strong anti-allergic activity against contact and delayed hypersensitivity in a mouse model.

The retrosynthetic analysis of these compounds is presented in Scheme 1. As one can observe, the key factors of this synthesis are the selection of protective groups of sugar and phenolic hydroxyl groups and the cyclization step of diol **3** to fused tricyclic compound **2**. Previously reported methodologies were applied to the synthesis of C-glycoside **3** from **4**[4] and the formation of flavone **1** from acetophenone derivative **2**.[5]

As shown in Scheme 2, we chose 3,4,6-tri-*O*-benzyl-D-glucal (**5**) as a starting material. After stereoselective




**Scheme 1.** Retrosynthetic analysis.

epoxidation[6] using dimethyldioxirane, followed by treatment with methanol, methyl 3,4,6-tri-*O*-benzyl-β-D-glucoside was obtained. For acid stability and a deprotective condition, *p*-nitrobenzyl ether was selected as a protective group[7] for the 2-position of this glucose derivative. After hydrolysis of methyl glucoside with dil $H_2SO_4$, then conversion to the corresponding glycosyl fluoride (**7**) using (diethylamino)sulfur trifluoride (DAST) at −78 °C,[8] C-glycosidation of **7** with 1-[2,4-bis(benzyloxy)-6-hydroxyphenyl]ethanone (**15**)[9] was examined using several reported methods,[4] and $BF_3OEt_2$/MS4A in $CH_2Cl_2$ gave the best results. Interestingly, the protective groups of both the glycosyl donor and acceptor greatly affected the C-glycosidation results. Among the hydroxyl protective groups of the 2-position

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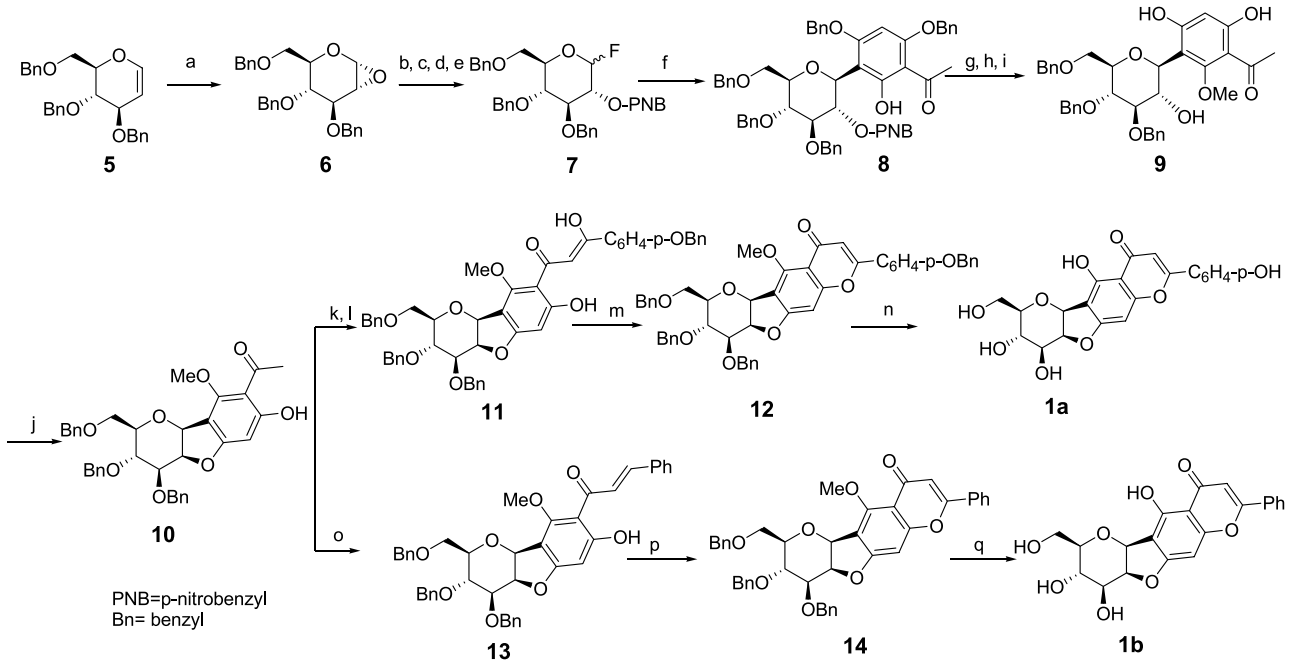


**Scheme 2.** Reagents and conditions: (a) dimethyldioxirane, $CH_2Cl_2$, 0 °C, quant; (b) MeOH/$CH_2Cl_2$, rt, quant.; (c) *p*-$NO_2$–$C_6H_4CH_2Br$, 2,4,6-collidine, AgOTf, 35 °C, 63%; (d) 2 M $H_2SO_4$, AcOH, reflux, 50%; (e) DAST, $CH_2Cl_2$, −78 °C, 78%; (f) 1-[2,4-bis(benzyloxy)-6-hydroxyphenyl]ethanone (**15**), $BF_3OEt_2$, MS4A, −20 °C, then rt, 56%; (g) MeI, NaH, DMF, rt, 86%; (h) indium powder, satd $NH_4Cl$, MeOH/*i*PrOH, 85 °C, 73%; (i) 10% Pd–C, THF, rt, 92%; (j) 1,1′-azobis(*N*,*N*-dimethylformamide), $Bu_3P$, benzene, rt, 73%; (k) *p*-benzyloxybenzoyl chloride, DMAP, $CH_2Cl_2$, rt, 88%; (l) LDA, THF, −30 °C, 50%; (m) TMSOTf, $CH_2Cl_2$, rt, 62%; (n) $BCl_3$, $CH_2Cl_2$, rt, then $NaHCO_3$, HPLC purification, 10%; (o) PhCHO, 50% NaOH, dioxane, rt, 50%; (p) $C_6H_5I(OAc)_2$, KOH/MeOH, rt, 52%; (q) $BCl_3$, $CH_2Cl_2$, rt, evaporation, then aq 1 M HCl–MeOH; rt, 3 days, 44%.

of **4**, such as *p*-nitrobenzyl ether, triethylsilyl ether, and methoxymethyl ether, only *p*-nitrobenzyl ether gave the desired product. Among the protective groups of 2,4,6-trihydroxyacetophenone, such as benzyl ether, *t*-butyldiphenylsilyl ether, and *t*-butyldimethylsilyl ether, only **15** gave the desired product **8**. After protection of the phenolic hydroxyl group of **8** by MeI and NaH, followed by deprotection of the *p*-nitrobenzyl group by indium–aqueous ammonium chloride,[5b] and deprotection of phenolic benzylether by Pd–C/$H_2$ in THF, **9** was obtained. With cyclization of triol (**9**) by the Mitsunobu condition,[10] the desired compound **10** was obtained in good yield. Using this key intermediate, the flavones **12** and **14** were synthesized in two different ways. The natural product precursor (**12**) was synthesized by acylation of 2′-OH by acyl chloride/DMAP, then rearrangement of the acyl group by a large excess of LDA, and finally, cyclization by TMSOTf.[3] On the other hand, **14** was prepared by condensation with benzaldehyde, followed by flavone formation with hypervalent iodine reagent.[5b] Debenzylation of **14** using Pd–C/$H_2$ in AcOEt did not give the desired deprotected compound but gave a furan ring-decomposed compound. To obtain the desired product, fully protected **12** or **14** were treated with $BCl_3$ in $CH_2Cl_2$ at room temperature for 5 min, then the reaction was quenched by aqueous $NaHCO_3$ or MeOH, respectively. Because boronic ester along with the desired product was obtained in the case of MeOH quenching, the crude reaction mixture was treated with aqueous 1N–HCl/MeOH at room temperature for 3 days to give the desired product **1b**. By HPLC purification, pure **1a** and **1b**[11] were obtained.

Oral administration of chemically synthesized **1b** reduced the skin reaction, which was elicited by an epicutaneous application of 2,4,6-trinitro-1-chlorobenzene (TNCB) to the ears of sensitized mice in a dose-dependent manner at 2 and 10 μg/kg, as shown in Figure 1.[12] Compared with natural product (**1a**), which reduced the increase of ear thickness by 30% at a dose of 10 μg/kg, **1b** showed stronger effect (59% reduction at the same dose). And compared with betamethasone, which was

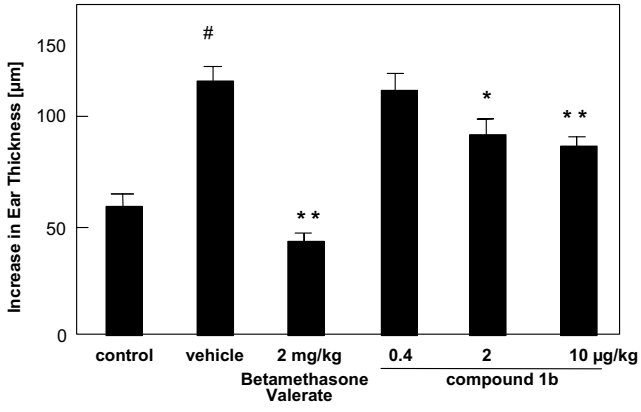


**Figure 1.** Effect of **1b** on contact hypersensitivity reaction in mice. Values are means ± SEM ($N = 7$). Contact hypersensitivity reaction was induced by topical application of TNCB to the ears of sensitized mice, and the skin reaction was determined at 24 h after the elicitation. (#) $P < 0.01$ compared with unsensitized control group (Student's *t*-test); (*) $P < 0.05$; (**) $P < 0.01$ compared with vehicle group (Dunnett's test).

administered orally at a dose of 2 mg/kg, the efficacious dose of **1b** was much lower.

In conclusion, we have demonstrated the first total synthesis of tricyclic flavonoid **1a** and its application to the synthesis of a new derivative **1b**, which showed a stronger anti-inflammatory effect than that of the natural flavonoid **1a**.

Further studies on the mechanistic aspects of the anti-inflammatory effects of these novel flavonoids are under investigation.

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11. $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ 3.3–3.4 (m, 1H), 3.55–3.65 (m, 2H), 3.82 (dd, 1H, $J = 3$ Hz, 12 Hz), 3.99 (dd, 1H, $J = 5$ Hz, 9 Hz), 4.7–4.75 (m, 1H), 5.22 (d, 1H, $J = 3$ Hz), 6.70 (s, 1H), 6.81 (s, 1H), 7.5–7.6 (m, 3H), 7.95–8.05 (m, 2H).
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