Hydrolysis of scutellarin and related glycosides to scutellarein and the corresponding aglycones

Wei Zhang, Dong Yi, Kai Gao, Manshuo Liu, Jian Yang, Xiali Liao* and Bo Yang

Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, P.R. China

Scutellarein has been prepared by the hydrolysis of scutellarin with sulfuric acid to provide this biologically important rare flavone in up to >90% yield and on the 5 g scale in minutes. This protocol has been applied to five other flavonoid glycosides (rutin, hesperidin, naringin, baicalin and diosmin) which are readily hydrolysed to their corresponding aglycones.

Keywords: scutellarein, scutellarin, flavonoid glycoside, hydrolysis, sulfuric acid

Scutellarein (5, 6, 7, 4′-tetrahydroxyflavone, SCUE, **1**, Fig. 1) is the aglycone of scutellarin (scutellarein-7-*O*-glucuronide, SCU, **2**, Fig. 1), and is the main active constituent of breviscapine, an extract from the Chinese traditional herb *Erigeron breviscapus* (vant.) Hand.-Mazz. Scutellarin, mostly in the forms of breviscapine, has been used in the clinic for the treatment of cardio- and cerebrovascular diseases for decades in China. However, it suffers badly from its poor solubility[1] and low oral bioavailability.[2,3] SCUE has better solubilities and much more superior absolute bioavailability than SCU *in vivo*.[4] Recent reports disclosed that SCUE was the main metabolite of SCU when it was administered to rats.[5–8] Moreover, it was reported that SCUE had other important physio- and pharmacological activities such as preventing vascular endothelial dysfunction,[9] inhibiting the proliferation of human retinal endothelial cells (HREC),[10] inhibiting SARS-CoV (Severe acute respiratory syndrome-coronavirus),[11] decreasing neurological deficit score and cerebral infarction volume,[12] and selective cytotoxicities towards cancer cells.[13] Hence SCUE is worth further development as a new lead compound or drug candidate.

However, SCUE is rare in nature in contrast to SCU. Total syntheses of SCUE have been reported by Farkas,[14] Wu[15] and Silvestri[16] respectively, but they all involved lengthy processes and gave low yields (all below 15%). In the light of the natural abundance of SCU,[17,18] the preparation of SCUE from SCU might provide a useful approach. Glycosidic bonds can be hydrolysed assisted by glycoside hydrolases. Alternatively, acidic hydrolysis of a glycoside represents a common chemical method to obtain its aglycone. Very recently, Li,[12,19,20] Tang[21] and Wang[22] independently examined the hydrolysis of SCU in aqueous mineral acid, but they all needed high temperature and long reaction time. The yields were not satisfactory (mostly below 20%). When we repeated these aqueous acidic hydrolysis procedures elaborate separation processes were required because of the very complex mixture of products. Consequently it is of value to develop a more efficient and practical process for the transformation of SCU to SCUE. We now disclose a simple yet efficient protocol for the synthesis of SCUE *via* the hydrolysis of SCU with sulfuric acid.

Results and discussion

In preliminary work, we found that we barely got any product by stirring a solution of SCU either dilute or concentrated sulfuric acid at room temperature. A considerable amount of heat is immediately generated when concentrated sulfuric acid comes in contact with water. Hence we conducted the hydrolysis with the assistance of this exothermic hydration. SCU was dissolved in concentrated sulfuric acid by shaking or ultrasonic agitation at room temperature and this was followed by the dropwise addition of a specific amount of water. Heat was released and yellow crystals began to form. The mixture was then allowed to stand for several minutes before a large volume of water was added with stirring. The yellow crystals that were deposited were collected by suction filtration. They were characterised spectroscopically as the target product.

The reaction conditions were then optimised as shown in Table 1. When sulfuric acid (1.5 mL, 98%, w/w) was used with milligram amounts of SCU (50 mg), an increase of the volume of the water that was added (from 1.5 to 3.0 mL) decreased the yield (from 84 to 76%, entries 1 and 2, Table 1). When the volume of sulfuric acid was adjusted to 2.0 mL followed by the equal amount of water (2.0 mL), the yield was raised to 93% (entry 3). Again an increase in the amount of water decreased the yield (entry 4). The use of more sulfuric acid (2.5 mL) reduced the yield significantly (entry 5).

With the optimised reaction conditions in hand, we increased the amount of the substrate using this established protocol. An excellent yield was obtained when the substrate was raised to 1.0 g scale (90%, entry 6, Table 1). Moreover, the amount of sulfuric acid that was used was reduced to 20 mL. When the method was used on the 5 g scale, it was found that the amount of sulfuric acid could even be reduced to 10 mL and still a satisfactory yield was obtained (87%, entry 7, Table 1). It seemed that the amount of sulfuric acid, which was just enough to dissolve the substrate, was adequate for the completion of the hydrolysis of the substrate by this procedure.

To explore the scope of this protocol, it was then used for the preparation of other flavonoid aglycones by hydrolysis

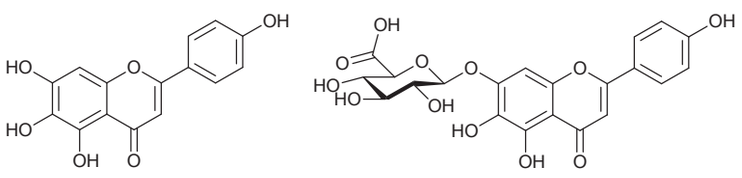
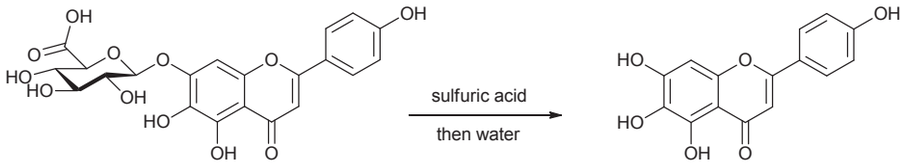


Fig. 1 Structures of scutellarein (SCUE, **1**) and scutellarin (SCU, **2**).

* Correspondent. E-mail: xlliao@yahoo.com

Table 1 Hydrolysis of SCU by sulfuric acid[a]



2 **1**

Entry	SCU/mg	Sulfuric acid /mL	Water/mL	Reaction time/ min	Yield/%[b]
1	50	1.5	1.5	10	84
2	50	1.5	3.0	10	76
3	50	2.0	2.0	10	93
4	50	2.0	4.0	10	83
5	50	2.5	2.5	10	74
6[c]	1000	20	20	15	90
7[d]	5000	10	20	15	87

[a]Reaction conditions: A beaker (100 mL) containing SCU (50 mg) was treated with the specified amounts of sulfuric acid (98%, w/w) and water sequentially at room temperature. When the hydrolysis was completed (followed by TLC), the mixture was added a larger volume of water (15 mL) with stirring. Yellow crystals that were deposited were collected by suction filtration and washed by water. Recrystallisation or column chromatography on silica gel could help if there were any impurities.
[b]Isolated yield.
[c]The volume of water used for precipitation of the product was 200 mL.
[d]The volume of water used for precipitation of the product was 300 mL.

of the corresponding natural glycosides. These flavones are considered to be of biological and pharmaceutical importance, but their natural sources are limited and their production by total synthesis was still impracticable. Their widely available flavanoid glycosides were selected to be hydrolysed to their aglycones under the established conditions. Fortunately, the hydrolysis proceeded smoothly and moderate to excellent yields were obtained on a 50 mg substrate scale (Table 2). This could provide a new general approach to the preparation of these biologically important flavones.

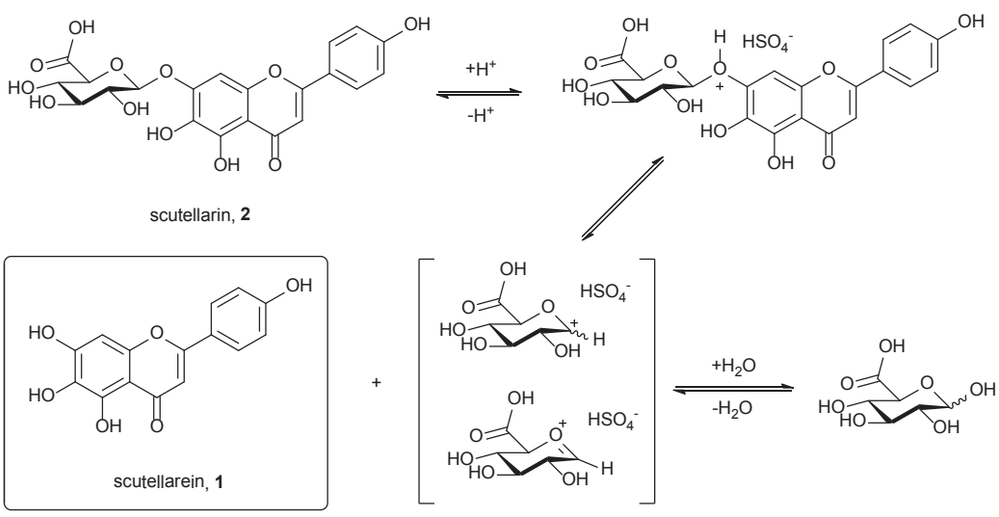
The possible reaction mechanism is shown in Scheme 1 with scutellarin hydrolysis as the example. The glycosidic oxygen was first protonated when the glycoside was dissolved in sulfuric acid. Second the glycosidic bond was cleaved to give an anomeric carbocation or its oxonium tautomer, and then the aglycone was deposited in water. The carbocation or oxonium ion was then discharged *via* a nucleophilic attack from water.

Table 2 Hydrolysis of flavonoid glycosides

Entry	Substrate	Product	Yield/%	M.p. (lit.)/°C
1	Rutin, **4**	Quercetin, **3**	89	310–313 (315)[23]
2	Hesperidin, **6**	Hesperetin, **5**	49	227 (220–222)[24]
3	Naringin, **8**	Naringenin, **7**	21	252 (247–250)[25]
4	Baicalin, **10**	Baicalein, **9**	93	260–262 (261–262)[26]
5	Diosmin, **12**	Diosmetin, **11**	85	256–258 (253–254)[24]

Conclusions

In summary, a simple and highly efficient protocol of the preparation of scutellarein from scutellarin has been developed. It was also successful in the hydrolysis of other flavonoid glycosides, leading to the corresponding biologically important flavones. This might provide a unique and practical approach to the preparation of the flavonoid aglycones. Further studies on the application of this protocol are in progress.



Scheme 1 Possible mechanism for glycoside hydrolysis with sulfuric acid

Experimental

Scutellarin was obtained from Kunming Pharmaceutical Corporation (KPC) in Kunming, China. The sulfuric acid (98%, w/w) was of analytical grade. The melting points were determined on an RY-1 instrument which was uncorrected. IR spectra were recorded on a Bio-Rad FTS-40 FT-IR spectrometer. NMR experiments were conducted on a Bruker Avance DRX 500 spectrometer or a Bruker Avance III 400 at 298 K, and the ESI-MS data were recorded on an Agilent 6210 mass spectrometer or a Bruker MicrOTOF Q-II mass spectrometer.

Typical hydrolysis procedure (for entry 3, Table 1)

Sulfuric acid (2.0 mL, 0.037 mmol) was added dropwise to a beaker (100 mL) containing scutellarin (50 mg, 0.11 mmol). It was shaken or agitated by ultrasound agitated to dissolve the substrate in the acid at room temperature. Water (2.0 mL) was then added carefully dropwise. When the evolution of heat ceased (in 10 minutes), the mixture was added to water (15 mL) in one portion with stirring with a glass rod. The yellow crystals that were deposited were collected by suction filtration and washed by water (5 mL). In most cases, such products were pure enough for direct use. Moreover, it could be further purified by recrystallisation from aqueous methanol (70%, v/v) or column chromatography on silica gel (eluent: ethyl acetate/formic acid/water = 100/4/3, v/v/v, R_fs of SCU and SCUE were 0.1 and 0.8 on silica gel GF_{254} respectively). Light yellow crystals were obtained after recrystallisation (28.5 mg, 93% yield); m.p. 285–287 °C (> 300 °C)[27]. IR (KBr, cm^{-1}): ν_{max} 3442, 3331, 3098, 1671, 1619, 1587, 1509. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.80 (s, 1H, 5-OH), 10.48 (s, 1H, 7-OH), 10.33 (s, 1H, 4′-OH), 8.75 (s, 1H, 6-OH), 7.92 (d, 2H, J = 8.6 Hz, C2′, C6′-H), 6.92 (d, 2H, J = 8.6 Hz, C3′, C5′-H), 6.75 (s, 1H, C3-H), 6.58 (s, 1H, C8-H). HR-ESI-MS (*m/z*): 309.0363 for $[M+Na]^+$, calcd 309.0370.

On a gram scale of substrate, a round-bottomed flask equipped with an efficient magnetic stirrer was more efficient for the experimental operation.

Hydrolysis process of other flavonoid glycosides: The procedures were all similar to the above mentioned. The substances that were used were as follows: glycoside (50 mg), sulfuric acid (2 mL), water (2 mL), then water for the product precipitation (15 mL). Reaction process was monitored by TLC (silica gel GF_{254}, eluent: ethyl acetate/formic acid/water = 100/4/3).

Quercetin (**3**): Light yellow powder; yield 89%; m.p. 310–313 °C (315 °C)[23]; IR (KBr, cm^{-1}): ν_{max} 3407, 1611, 1520, 1263; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.51 (s, 1H), 10.82 (s, 1H), 9.63 (s, 1H), 9.40 (s, 1H), 9.34 (s, 1H), 7.68 (d, J = 2.2 Hz, 1H), 7.55 (dd, J = 8.5, 2.2 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.41 (d, J = 2.0 Hz, 1H), 6.19 (d, J = 2.0 Hz, 1H). HRESI-MS (*m/z*): 301.0357 for $[M-H]^-$, calcd 301.0348.

Hesperetin (**5**): Yellow powder; yield 49%; m.p. 227 °C (220–222 °C)[24]; IR (KBr, cm^{-1}): ν_{max} 3500–3300, 2947, 1638, 1593, 1459, 1235; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.15 (s, 1H), 10.84 (s, 1H), 9.10 (s, 1H), 6.95–6.83 (m, 3H), 5.91–5.82 (dd, J = 5.3, 1.7 Hz, 2H), 5.43 (dd, J = 9.9, 2.4 Hz, 1H), 3.76 (s, 3H), 3.24–3.16 (dd, J = 13.7, 9.9 Hz, 1H), 2.68 (dd, J = 13.7, 2.5 Hz, 1H); HRESI-MS (*m/z*): 301.0714 for $[M-H]^-$, calcd 301.0712.

Naringenin (**7**): Yellow powder; yield 21%; m.p. 252 °C (247–250 °C)[25]; IR (KBr, cm^{-1}): ν_{max} 3136, 1639, 1603, 1493. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.15 (s, 1H), 10.80 (s, 1H), 9.60 (s, 1H), 7.71 (m, 2H), 7.66 (m, 2H), 5.87 (s, 2H), 5.48–5.37 (m, 1H), 3.27 (dd, J = 17.2, 12.9 Hz, 1H), 2.66 (dd, J = 17.0, 3.0 Hz, 1H).

Baicalein (**9**): Yellow powder; yield 93%; m.p. 260–262 °C (261–262 °C)[26]; IR (KBr, cm^{-1}): ν_{max} 3560, 1727, 1623, 1583; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.65 (s, 1H), 10.59 (s, 1H), 8.83 (s, 1H), 8.06 (dd, J = 10.8, 4.0 Hz, 2H), 7.58 (m, 3H), 6.94 (s, 1H), 6.62 (s, 1H).

Diosmetin (**11**): Yellow powder; yield 85%; m.p. 256–258 °C (253–254 °C)[24]; IR (KBr, cm^{-1}): ν_{max} 3500–3200, 2942, 1658, 1612, 1437, 1259; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.93 (s, 1H), 10.87 (s, 1H), 9.49 (s, 1H), 7.54 (dd, J = 8.6, 2.3 Hz, 1H), 7.42 (d, J = 2.2 Hz, 1H), 7.09 (d, J = 8.6 Hz, 1H), 6.76 (s, 1H), 6.46 (d, J = 2.1 Hz, 1H), 6.19 (d, J = 2.1 Hz, 1H), 3.85 (s, 3H); HRESI-MS (*m/z*): 299.0547 for $[M-H]^-$, calcd 299.0556.

This study was financially supported by Natural Science Foundation of Yunnan Province (No. 2011FZ059, to X. Liao) and National Natural Science Foundation of China (Nos. 21062009 and 21362016, to B. Yang). The authors thank KPC for kind donation of scutellarin, and the editor for suggestions on the hydrolysis mechanism.

Received 12 February 2014; accepted 7 May 2014
Paper 1402462 doi: 10.3184/174751914X14017253941699
Published online: 12 July 2014

References

1 L.-J. Luan, Y. Zhou, Y.-J. Wu, X.-S. Liu and L.-H. Wang, *J. Chem. Eng. Data*, 2010, **55**, 5299.
2 Q.H. Ge, Z. Zhou, X.J. Zhi, L.L. Ma and X.H. Chen, *Chin. J. Pharm.*, 2003, **34**, 618. (in Chinese).
3 J.-F. Xing, H.-S. You, Y.-L. Dong, J. Lu, S.-Y. Chen, H.-F. Zhu, Q. Dong, M.-Y. Wang and W.-H. Dong, *Acta Pharmacol. Sin.*, 2011, **32**, 655.
4 Q.M. Che, L.Y. Pan, Y. Chen and H. He, *Chin. Pharm. J.*, 2007, **42**, 1418. (in Chinese).
5 J.L. Zhang, Q.M. Che, S.Z. Li and T.H. Zhou, *J. Asian Nat. Prod. Res.*, 2003, **5**, 249.
6 X.R. Ran, S.Z. Si, Q.L. Liang and A. Luo, *Chin. Chem. Lett.*, 2006, **17**, 1225.
7 F. Qiu, H.J. Xia, T.Y. Zhang, X. Di, G.X. Qu and X.S. Yao, *Planta Med.*, 2007, **73**, 363.
8 H.J. Xia, F. Qiu, S. Zhu, T.Y. Zhang, G.X. Qu and X.S. Yao, *Biol. Pharm. Bull.*, 2007, **30**, 1308.
9 B.-H. Zhu, Y.-Y. Guan, H. He and M.-J. Lin, *Acta Pharmacol. Sin.*, 2000, **21**, 353.
10 R. Gao, B.-H. Zhu, S.-B. Tang, J.-F. Wang and J. Ren, *Acta Pharmacol. Sin.*, 2008, **29**, 707.
11 M.S. Yu, J. Lee, J.M. Lee, Y. Kim, Y.-W. Chin, J-G. Jee, Y.-S. Keum and Y.-J. Jeong, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 4049.
12 L. Qian, M. Shen, H. Tang, Y. Tang, L. Zhang, Y. Fu, Q. Shi and N.-G. Li, *Molecules*, 2012, **17**, 10667.
13 V. Chen, R.E. Staub, S. Baggett, R. Chimmani, M. Tagliaferri, I. Cohen and E. Shtivelman, *PloS ONE*, 2012, **7**, e30107.
14 L. Farkas, G. Mezey-Vándor and M. Nógrádi, *Chem. Ber.*, 1974, **107**, 3878.
15 J.M. Cui, G. Fang, Y.B. Duan, Q. Liu, L.P. Wu, G.H. Zhang and S. Wu, *J. Asian Nat. Prod. Res.*, 2005, **7**, 655.
16 G. Righi, I.P. Silvestri, M. Barontini, F. Crisante, A.D. Manno, R. Pelagalli and P. Bovicelli, *Nat. Prod. Res.*, 2012, **26**, 1278.
17 Y.Z. Li, W.Y. Liu, G.D. Li, G.X. Han and W.D. Zhang, *Chin. J. Pharm.*, 2002, **33**, 281. (in Chinese).
18 M. Gao, W. Huang and C.Z. Liu, *J. Chrom. B*, 2007, **858**, 22.
19 N.-G. Li, S.-L. Song, M.-Z. Shen, Y.-P. Tang, Z.-H. Shi, H. Tang, Q.-P. Shi, Y.-F. Fu and J.-A. Duan, *Bioorg. Med. Chem.*, 2012, **20**, 6919.
20 N.-G. Li, M.-Z. Shen, Z.-J. Wang, Y.-P. Tang, Z.-H. Shi, Y.-F. Fu, Q.-P. Shi, H. Tang and J.-A. Duan, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 102.
21 L.H. Qian, N.-G. Li, Y.-P. Tang, L. Zhang, H. Tang, Z.-J. Wang, L. Liu, S.-L. Song, J.-M. Song, J.M. Guo and A.W. Ding, *Int. J. Mol. Sci.*, 2011, **12**, 8208.
22 X.Z. Fu, F.J. Xing, Y.Y. Lan, A. Wang, Y. Wang, J. Li, W. Zhou, W. Zhang and Y. Liu, *Chin. J. Org. Chem.*, 2011, **31**, 1043. (in Chinese).
23 L. Wang, X. Li, S. Zhang, W. Lu, S. Liao, X. Liu, L. Shan, X. Shen, H. Jiang, W. Zhang, J. Huang and H. Li, *Bioorg. Med. Chem. Lett.*, 2012, **20**, 4164.
24 M. Correia-da-Silva, E. Sousa, B. Duarte, F. Marques, F. Carvalho, L.M. Cunha-Ribeiro and M.M.M. Pinto, *J. Med. Chem.*, 2011, **54**, 95.
25 N.I. Kulesh, N.A. Vasilevskaya, M.V. Veselova, V.A. Denisenko and S.A. Fedoreev, *Chem. Nat. Comp.*, 2008, **44**, 712.
26 R. Bhuyan and C.N. Saikia, *Bioresource Tech.*, 2005, **96**, 363.
27 H. Gao, J. Kawabata, *Biosci. Biotechnol. Biochem.*, 2004, **68**, 1858.