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단신 (Notes)

Rapid Separation of Gossypol from Cottonseeds by a Solid-Phase Synthetic Method

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The structure of gossypol was determined as 1,1',6,6',7,7'-hexahydroxyl-5,5'-diiso-propyl-3,3'-dimethyl-(2,2'-binaphthalene)-8,8'-dicarboxaldehyde (*Fig.* 1).[1] It is a well-known natural compound due to its contraceptive activity.[2] An original antiviral medicine called Kagocel, which is a polymeric structure based on gossypol linked with cellulose, is developed and produced by NEARMEDIC company.[3] The medicine is not absorbed by gastrointestinal tract, so it does not enter internal fluids of the body, which makes the medicine non-toxic and safe. The gossypol based polymeric drug Kagocel has been proven to be effective in numerous clinical tests for the treatment and prevention of influenza and herpes infections in adults and children.[4–6] Total synthesis of gossypol has been achieved,[7] and many methods for extraction and separation have been applied to purify it.[8–11] The shortcomings of these preparation methods include large requirements of time, high-cost, and the usage of toxic solvents. The chemical structure of gossypol is unstable as gossypol is air and light sensitive, so it is urgent to find a rapid method to purify it for the purpose of medical use or futher research. In our present investigation, the optimal extraction conditions for crude gossypol such as solvent and time were selected, and a solid-phase synthetic method included formation of a Schiff base and followed by hydrolysis has been successfully developed to separate high-quality gossypol (*Fig.* 2). The

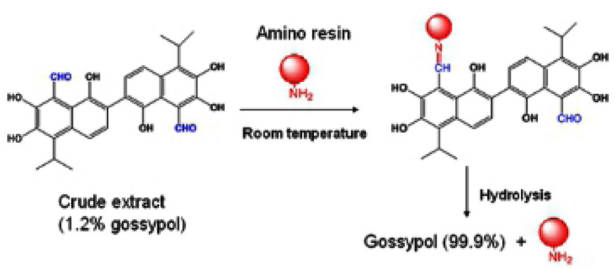

***Figure* 1.** Structure of gossypol and synthetic method.

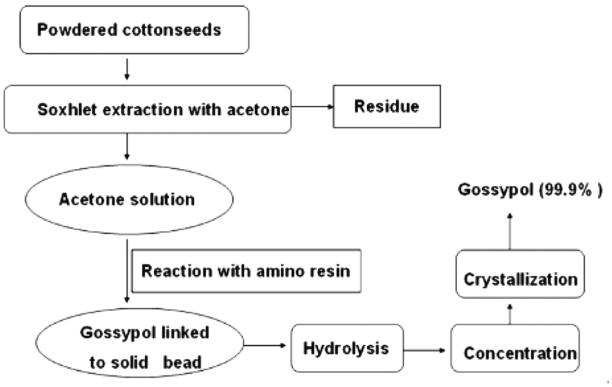

***Figure* 2.** Process for the production of gosssypol.

purity of gossypol obtained in each step was determined and compared with the reference sample. The purity of the final product was 99.9% as determined by HPLC.

EXPERIMENTAL

Apparatus

The high-performance liquid chromatography equipment (Waters 600, Maple Street Milford, Massachusetts, USA) used was a system including a Waters 600 pump, and a Waters 2487 dual absorbance detector. The analysis was carried out with a Hypersil ODS column (250 mm × 4.6 mm, I.D., 5 μm; Dalian, China). Evaluation and quantification were made on a N2000 workstation (Hangzhou, Zhejiang University, China).

Reagents and materials

All organic solutions used for the extraction were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Methanol used for HPLC was of HPLC-grade and purchased from Fisher Scientific

Company (Fair Lawn, NJ, USA). Reference gossypol (98% purity) was purchased from Xi'an Kailai Bioengineering Co., Ltd (Xi'an, China). The cottonseeds were collected from Huaibei, Anhui Province of China in June, 2013 and were identified by Prof. Weihua Yang, Institute of Cotton Research, Chinese Academy of Agriculture Sciences. A voucher specimen (Code: 20130606) is deposited at the College of Chemistry and Chemical Engineering, Anyang Normal University.

Solid-phase adsorbent and pretreatment

Macroporous adsorption resin (D380) is an amino resin, which was purchased from The Chemical Plant of NanKai University (Tianjin, China). It was pretreated with 2% NaOH solution, deionized water, 5% HCl solution and deionized water successively to remove the monomers and porogenic agents trapped inside the pore during the synthetic process. Prior to use, the resin was put into a Buchner funnel and washed with deionized water followed by acetone, and then dried by vacuum suction.

Preparation of standard solutions

A 5.0 mg sample of accurately weighed gossypol standard was introduced into a 50 mL volumetric flask and made up to the volume with methanol as a working calibration solution (100 mg L^{-1}). Aliquots of 3, 5, 7, 10 and 12 mL working calibration solutions were injected for HPLC analysis, and the peak areas were 32007.8, 53372.2, 79725.5, 111718.4 and 135483.4, respectively. The calibration curve was determined as y = 115202x–2787.9 (R = 0.9995).

Solvent selection for preparation of crude extract

Due to the highly lipophilic property of gossypol, both free and protein-bound gossypol is present within cottonseeds. It is necessary to choose a proper solvent for the extraction of gossypol. In this study, methanol, ethanol, dichloromethane, ethyl acetate, acetone, and a mixture of dichloromethane and methanol (1:1, v/v) were used to extract gossypol. The extraction kinetics for each solvent was measured as follows. The powdered cottonseeds (20.0 g) were wrapped with a piece of filter paper and then put into a Soxhlet extractor. Then 150.0 mL of appropriate solvent was added and the mixture was heated to reflux for 12 h. The concentration of gossypol in the extractor was determined every 2 h by HPLC.

Solid-phase synthesis

Gossypol has two aldehyde groups which allow the molecule to bind to a bead even at room temperature to form a Schiff's base. Therefore, a type of macroporous exchange resin (D380) was selected to link gossypol to the bead. The binding kinetics curve of gossypol on to the resin was performed as follows: acetone extraction of cottonseeds (150 mL) and pretreated resin (20 g) were stirred in a 250 mL round-bottomed flask for 4 h at room temperature, and the content of gossypol in solution was determined every 20 minutes by HPLC.

Dynamic desorption test (hydrolysis)

Hydrolysis tests were carried out as follows: the Schiff's base resin (20 g) was filtered and washed several times with petroleum ether and acetone successively to remove unbound substances. Then the resin was transferred into a 250 mL flask and a mixture solvent of 60 mL acetone-acetic acid (1:1, v/v) was added, followed by addition of 5 mL of 9% H_2SO_4. The mixture was stirred at room temperature over night, and the content of gossypol was analyzed every 2 h by HPLC.

RESULTS AND DISCUSSION

Selection of solvent and reflux time for the extraction

As showed in *Fig.* 3, several solvents were tested for the extraction of gossypol from cottonseeds. Acetone was the most suitable solvent for the extraction process, it was more efficient than dichloromethane, ethyl acetate, ethanol, methanol, or the mixture of methanol and dichloromethane (1:1). The possible reason is that acetone has a low viscosity, moderate boiling point, highly penetrability to the powdered tissues of cottonseeds and good solubility for gossypol. The optimal extraction time was determined as 12 h, and after this point the content of gossypol did not increase. It was interesting to note that the content of gossypol decreased after 10 h if dichloromethane or ethyl acetate was used as the extraction solvent. Therefore, acetone was selected as the optimal solvent.

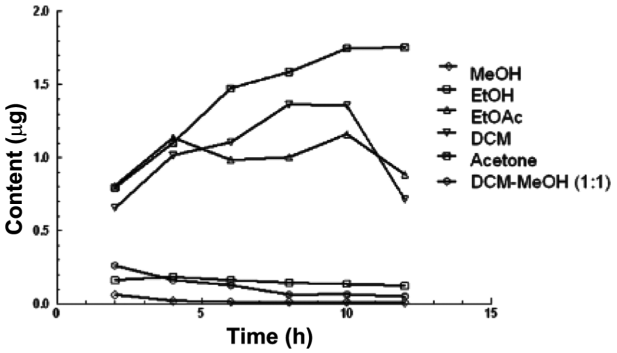

***Figure* 3.** Influence of solvents and time on extraction.

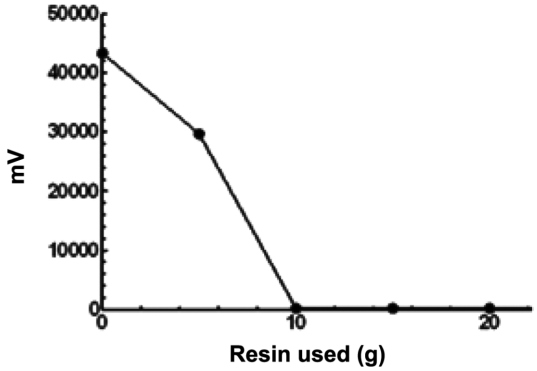

***Figure* 4.** The adsorption capacity of D380 resin.

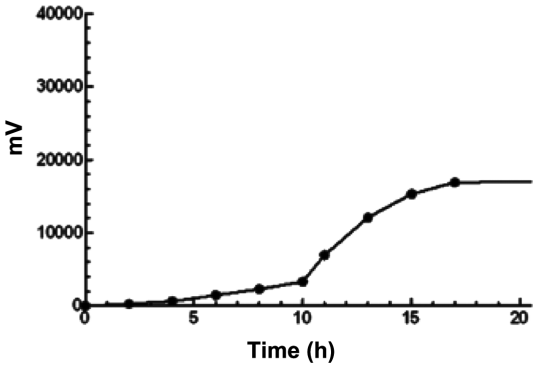

***Figure* 6.** Hydrolysis time of the gossypol-Schiff's base.

Binding capacity of the amino resin

In order to determine the adsorption capacity of D380 resin, 5, 10, 15, 20, 25 g of pretreated D380 resin were put into five 250 mL flat-bottom flasks, and allowed to react with 50 mL of acetone extract (gossypol content was 1.6 mg mL^{-1}) for 4 h. The concentration of gossypol in each flask was measured by HPLC. The results (*Fig.* 4) demostrated that 10 g resin was adequate to adsorb the entire gossypol in the sample solution. Thus, the adsorption capacity of D380 resin was 8.0 mg/g.

Solid-phase synthesis of gossypol-resin Schiff base

The formation of gossypol-resin Schiff base was rapid and catalyst free, *Fig.* 5 shows that in the first 20 minutes, the concentration of free gossypol decreased significantly, as more than half of gossypol was reacted with the resin and bond to the bead. After 4 h of stirring at room temperature, the reaction was finished and the content of free gossypol no longer decreased.

Hydrolysis of gossypol - Schiff base

Hydrolysis of the gossypol-resin Schiff base was conducted in acidic solution, using a solution of weak sulfuric acid (0.69%). Gossypol was unstable at higher temperatures in acidic media, and all hydrolysis reactions were performed at room temperature. *Fig.* 6 shows that the concentration of gossypol does not increase from 18 h to 20 h, the reaction takes 18 h.

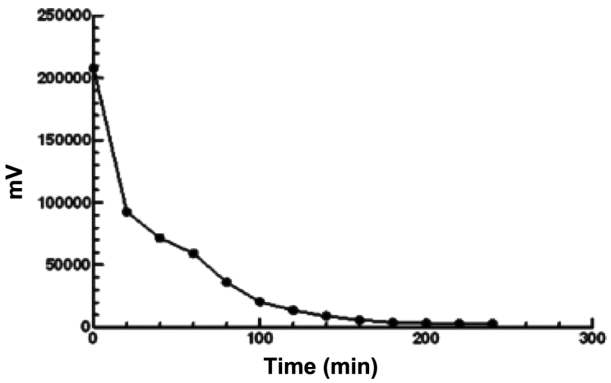

***Figure* 5.** Reaction time of gossypol with the amino resin.

Product recovery

After hydrolysis, the mixture was filtered with a Buchner funnel, and the resin was washed with acetone, allowing the resin to be reused. The filtrate was concentrated in vacuo at 55 °C to remove most of acetone and then put in a refrigirater (4 °C) over night. The precipitate was obtained by filtration and washed with distillated water. After drying in vacuo, the purity of gossypol was analyzed by HPLC to be 99.9% as shown in *Fig.* 7B. A comparison HPLC trace of crude acetone extract of cottonseeds can be found in *Fig.* 7A. To scale up this process, we have carried out three

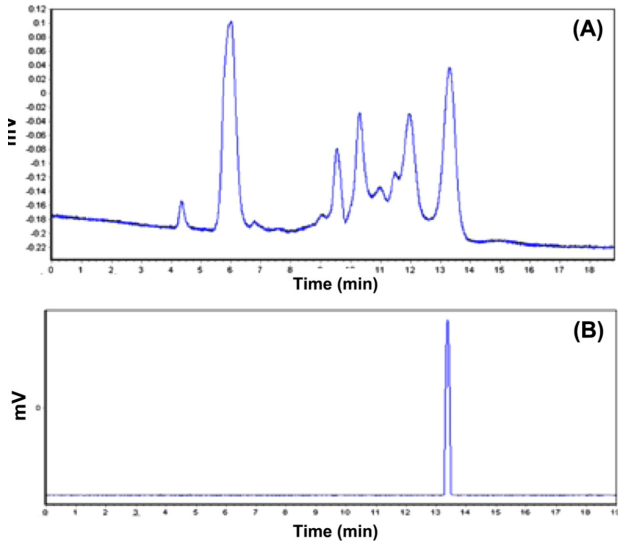

***Figure* 7.** HPLC chromatograms of the crude extract (A) and purified gossypol (B).

experiments at different scales following this method. In detail, from 20, 100 and 1000 g of cottonseeds raw material, 163, 840 and 9570 mg of gossypol have been obtained respectively in high purity, and the over all yield of gossypol was determined to be 0.85%, 0.84% and 0.96% respectively.

CONCLUSIONS

A rapid method to separate gossypol from cottonseeds has been developed. Powdered cottonseeds were extracted with acetone (7.5 mL per gram) using a Soxhlet extractor by heating under reflux for 12 h. The acetone solution was stirred with an amino resin at room temperature for 4 h to form a Schiff's base complex, which was then hydrolyzed with sulfuric acid (0.69%) at room temperature for 18 h to obtain gossypol in 99.9% purity. Furthermore, the amino resin bead can be recycled. The separation process was simple, fast, and economic. The results demonstrate that D380 amino resin is a promising candidate for large-scale separation and purification of gossypol from cottonseeds.

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REFERENCES

1. Campbell, K. N.; Morris, R. C.; Adams, R. *J. Am. Chem. Soc.* **1937**, *59*, 1723.
2. Hervé, J. C.; Pluciennik, F.; Bastide, B.; Cronier, L.; Verrecchia, F.; Malassiné, A.; Joffre, M.; Délèze, J. *Eur. J. Pharmacol.* **1996**, *313*, 243.
3. Galegov, G. A.; Narovlianskiĭ, A. N.; Sarymsakov, A. A.; Mezentseva, M. V.; Polonskiĭ, V. O.; Gomes, L. A.; Nesterenko, V. G.; Ershov, F. I. *Vopr. Virusol.* **2002**, *47*, 42.
4. Silin, D. S.; Lyubomska, O. V.; Ershov, F. I.; Frolov, V. M.; Kutsyna, G. A. *Curr. Pharm. Design.* **2009**, *15*, 1238.
5. Ershov F. I. *Zh Mikrobiol. Epidemiol. Immunobiol.* **2007**, *4*, 100.
6. Tazulakhova, E. B.; Parshina, O. V.; Guseva, T. S.; Ershov, F. I. *J. Interferon Cytokine Res.* **2001**, *21*, 65.
7. Cashaw, J. L.; Edwards, J. D., Jr. *J. Am. Chem. Soc.* **1957**, *79*, 2283.
8. Dowd, M. K.; Pelitire, S. M. *Ind. Crop. Prod.* **2001**, *14*, 113.
9. Dowd, M. K.; Pelitire, S. M. *J. Agric. Food Chem.* **2006**, *54*, 3265.
10. Halverson, J. O.; Smith, F. H. *Ind. Eng. Chem. Anal. Ed.* **1937**, *9*, 516.
11. Halverson, J. O.; Smith, F. H. *Ind. Eng. Chem. Anal. Ed.* **1934**, *6*, 356.