Journal of Organic Chemistry Research 有机化学研究, 2017, 5(2), 94-99
Published Online June 2017 in Hans. http://www.hanspub.org/journal/jocr
https://doi.org/10.12677/jocr.2017.52012

Hans汉斯

# Study on the Secondary Metabolites of *Aspergillus ochraceus*

**Qiongyu Zou[1], Haifeng Wu[2], Jun Huang[1], Li Zhang[1], Dizhao Cheng[1*], Xin Li[1], Guifei He[1], Fenfang Liang[1]**

[1]Key Laboratory of Hunan Province for Study and Utilization of Ethnic Medicinal Plant Resources, Department of Chemistry & Chemical Engineering, Huaihua University, Huaihua Hunan
[2]Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing
Email: *dzchen2002@163.com

Received: May 11th, 2017; accepted: Jun. 2nd, 2017; published: Jun. 5th, 2017

## Abstract

**Objective: To study the secondary metabolites from *Aspergillus ochraceus*. Methods: The constituents were isolated and purified by column chromatography and preparative TLC. Their structures were identified on the basis of comprehensive spectroscopic methods including ESI-MS and spectral data (1H, 13C-NMR). Results: Nine compounds were isolated and identified as ochratoxin A (1), ochratoxin B (2), neohydroxyaspergillic acid (3), (3R)-5-hydroxymellein (4), 5,6-dihydropenicillic acid (5), mesaconic acid (6), p-hydroxybenzoic acid (7), circumdatin G (8), (22E,24R)-ergosta-7,22-diene-3*β*,5*α*,6*β*-triol (9). Conclusion: The compounds 3~9 were isolated from *Aspergillus ochraceus* for the first time.**

## Keywords

***Aspergillus ochraceus*, Ochratoxin, Isolation and Identification**

# 赭曲霉次生代谢产物研究

**邹琼宇[1]，吴海峰[2]，黄　俊[1]，张　莉[1]，陈迪钊[1*]，李　欣[1]，贺贵妃[1]，梁芬芳[1]**

[1]湖南省怀化学院，民族药用植物资源研究与利用湖南省重点实验室，化学与化学工程系，湖南 怀化
[2]中国医学科学院北京协和医学院，药用植物研究所，中草药物质基础与资源利用教育部重点实验室，北京
Email: *dzchen2002@163.com

收稿日期：2017年5月11日；录用日期：2017年6月2日；发布日期：2017年6月5日
*通讯作者。

文章引用: 邹琼宇, 吴海峰, 黄俊, 张莉, 陈迪钊, 李欣, 贺贵妃, 梁芬芳. 赭曲霉次生代谢产物研究[J]. 有机化学研究, 2017, 5(2): 94-99. https://doi.org/10.12677/jocr.2017.52012

## 摘　要

**目的：研究赭曲霉次生代谢产物。方法：利用多种色谱层析方法分离出次生代谢产物，通过ESI-MS/MS及NMR等波谱方法鉴定结构。结果：共分离鉴定出9种化合物，依次是赭曲霉毒素A(1)、赭曲霉毒素B(2)、neohydroxyaspergillic acid(3)、(3R)-5-hydroxymellein(4)、5,6-dihydropenicillic acid(5)、mesaconic acid(6)、p-hydroxybenzoic acid(7)、circumdatin G(8)、(22E,24R)-ergosta-7,22-diene-3$\beta$,5$\alpha$,6$\beta$-triol(9)。结论：化合物3~9为首次从赭曲霉中分离得到的化合物。**



## 关键词

**赭曲霉，赭曲霉毒素，分离鉴定**




## 1. 引言

赭曲霉毒素具有肾毒、致癌、致畸、免疫抑制等毒性，主要由赭曲霉和青霉菌产生，包括赭曲霉毒素 A、B、C、D 等，其中赭曲霉毒素 A 的毒性最强[1] [2] [3] [4]。我们筛选了赭曲霉高产菌株 *Aspergillus ochraceus* 53216，通过大米发酵培养获得培养物，应用各种硅胶柱层析、反相中压色谱和半制备高效液相色谱分离得到 15 个化合物，采用现代波谱方法鉴定了其中的 9 个化合物，包括赭曲霉毒素 A(**1**)、赭曲霉毒素 B(**2**)、neohydroxyaspergillic acid(**3**)、(3*R*)-5-hydroxymellein(**4**)、5,6-dihydropenicillic acid(**5**)、mesaconic acid(**6**)、*p*-hydroxybenzoic acid(**7**)、circumdatin G(**8**)、(22E,24R)-ergosta-7,22-diene-3$\beta$,5$\alpha$,6$\beta$-triol(**9**)。结构式见图 1。

## 2. 结果与讨论

### 化合物结构鉴定

赭曲霉毒素 A(Ochratoxin A, OTA)(**1**)ESI-MS: *m/z* 426 $[M + Na]^+$, 分子式为 $C_{20}H_{18}ClNO_6$; $^1H$ NMR ($CDCl_3$, 600MHz) $\delta$: 8.38 (1H, s, H-6), 7.22-7.31 (5H, m, H-16~20), 4.88 (1H, m, H-13), 4.76 (1H, m, H-3), 3.30-3.34 (1H, m, H-14a), 3.30 (1H, m, H-4a), 3.16-3.20 (1H, m, H-14b), 2.82 (1H, m, H-4b), 1.58 (3H, d, $J$ = 6.0 Hz, H-21); $^{13}C$ NMR ($CDCl_3$, 150MHz) $\delta$: 174.4 (C-22), 169.8 (C-1), 162.7 (C-11), 159.0 (C-8), 140.8 (C-5), 138.8 (C-6), 136.8 (C-15), 129.5 (C-16,20), 128.5 (C-17,19), 127.0 (C-18), 123.2 (C-10), 120.8 (C-7), 110.2 (C-9), 76.0 (C-3), 55.1 (C-14), 37.8 (C-14), 32.4 (C-4), 20.7 (C-21)。以上数据与文献[5]报道的一致，故确定该化合物为赭曲霉毒素 A。

赭曲霉毒素 B(Ochratoxin B, OTB)(**2**)ESI-MS: *m/z* 370 $[M + H]^+$, 392 $[M + Na]^+$, 761 $[2M + Na]^+$, 分子式为 $C_{20}H_{19}NO_6$; $^1H$ NMR ($CDCl_3$，600 MHz) $\delta$: 8.32 (1H, d, $J$ = 7.8 Hz, H-7), 7.21~7.29 (5H, m, H-17~21), 6.83 (1H, d, $J$ = 8.4 Hz, H-6), 4.99 (1H, m, H-3), 4.76 (1H, td, $J$ = 6.0, 4.2 Hz, H-14), 3.32 (1H, dd, $J$ = 14.4, 5.4 Hz, H-15b), 3.19 (1H, $J$ = 13.8, 6.6 Hz, H-15a), 2.99 (1H, m, H-4b), 2.97 (1H, m, H-4a), 1.55 (3H, d, $J$ = 6.0 Hz, H-11); $^{13}C$ NMR ($CDCl_3$，150MHz) $\delta$: 173.4 (C-22), 170.3 (C-1), 164.2 (C-12), 160.4 (C-9), 143.9 (C-5),

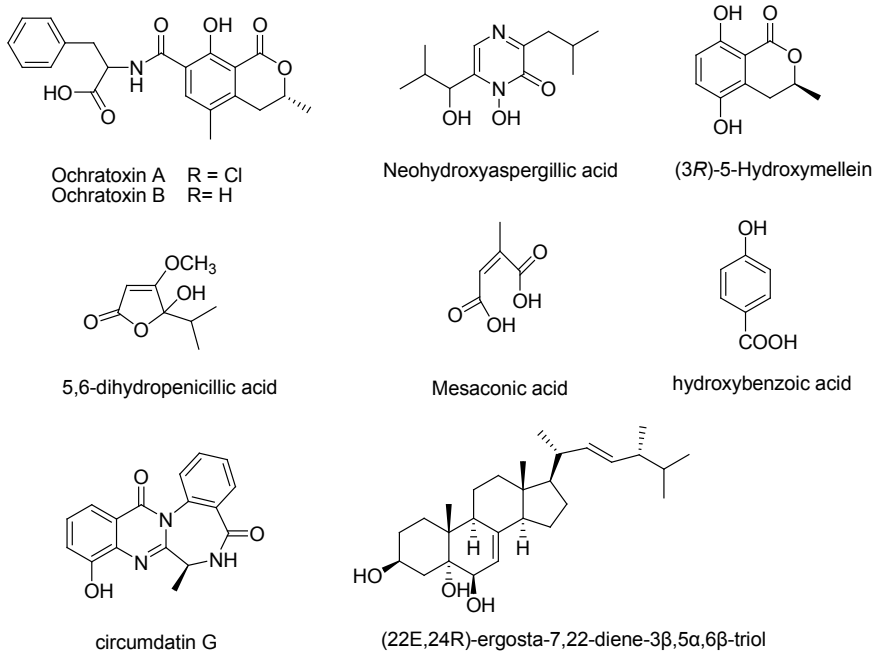


**Figure 1.** Compounds isolated from ferment of *Aspergillus ochraceus* 53216
**图 1.** 从 *Aspergillus ochraceus* 53216 发酵培养物中分离得到的化合物

138.9 (C-16), 136.4 (C-7), 129.5 (C-17,21), 128.6 (C-18,20), 127.1 (C-19), 119.2 (C-6), 118.7 (C-8), 108.8 (C-10), 76.4 (C-3), 54.3 (C-14), 37.7 (C-15), 34.7 (C-4), 20.7 (C-11)。以上数据与文献[6]报道的一致，故确定该化合物为赭曲霉毒素 B。

neohydroxyaspergillic acid(**3**)ESI-MS: *m/z* 241 $[M + H]^+$，263 $[M + Na]^+$，239 $[M - H]^-$，479 $[2M - H]^-$，分子式 $C_{12}H_{20}N_2O_3$; $^1$H NMR ($CDCl_3$, 600MHz) $\delta$: 7.56 (1H, s, H-5), 4.61 (1H, d, $J$ = 6.6 Hz, H-1″), 2.74 (2H, d, $J$ = 7.2 Hz, H-1′), 2.28 (1H, m, H-2″), 2.22 (1H, m, H-2′), 1.04 (3H, d, $J$ = 7.2 Hz, H-3″), 0.95 (9H, d, $J$ = 6.6 Hz, H-3′, 4′, 4″); $^{13}$C NMR ($CDCl_3$, 150MHz) $\delta$: 152.9 (C-2), 151.5 (C-3), 136.7 (C-6), 124.4 (C-5) , 73.8 (C-1″), 42.0 (C-1′), 32.4 (C-2″), 27.3 (C-2′), 22.7 (C-3′,4′), 19.6 (C-3″), 17.5 (C-4″). 以上数据与文献[7] [8]报道的一致，故确定该化合物为 neohydroxyaspergillic acid。

(3*R*)-5-hydroxymellein(**4**)ESI-MS: *m/z* 195 $[M + H]^+$，分子式 $C_{10}H_{10}O_4$; $^1$H NMR (DMSO-$d_6$, 600MHz) $\delta$: 10.39 (1H, s, 5-OH), 9.33 (1H, s, 8-OH), 7.06 (1H, d, $J$ = 9.0 Hz, H-7), 6.72 (1H, d, $J$ = 8.4 Hz, H-6), 4.73 (1H, m, H-3), 3.07 (1H, dd, $J$ = 16.8, 3.6 Hz, H-4a), 2.60 (1H, dd, $J$ = 16.8, 11.4 Hz, H-4b), 1.42 (3H, d, $J$ = 6.6 Hz, 3-$CH_3$); $^{13}$C NMR (DMSO-$d_6$, 150MHz) $\delta$: 169.5 (C-4), 153.9 (C-5), 145.5 (C-8), 124.5 (C-9), 123.9 (C-6), 115.1 (C-7), 108.0 (C-10), 75.9 (C-2), 28.0 (C-1), 20.4 (2-$CH_3$). 以上数据与文献[9] [10]报道的一致，故确定该化合物为 3*R*-5-Hydroxymellein。

5,6-dihydropenicillic acid(**5**)ESI-MS: *m/z* 195 $[M + Na]^+$, 155 $[M + H–H_2O]^+$，分子式 $C_8H_{12}O_4$; $^1$H NMR ($CD_3OD$, 600MHz) $\delta$: 5.02 (1H, s, H-2), 4.83 (3H, 3-$OCH_3$), 2.12 (1H, m, H-5), 1.02, 0.91 (6H, d, $J$ = 7.2 Hz, 2×$CH_3$); $^{13}$C NMR ($CD_3OD$, 150MHz) $\delta$: 182.4 (C-3), 173.7 (C-1), 107.5 (C-4), 90.3 (C-2), 60.6 (3-$OCH_3$), 49.6 (C-5), 34.9 (C-6,7). 以上数据与文献[11] [12]报道的一致，故确定该化合物为 5,6-dihydropenicillic acid。

Mesaconic acid(**6**)$^1$H NMR (DMSO-$d_6$, 600MHz) $\delta$: 10.98 (-OH), 10.56 (OH), 7.24 (1H, s), 1.72 (3H, s, $CH_3$); $^{13}$C NMR (DMSO-$d_6$, 150MHz) $\delta$: 164.9, 151.4, 137.6, 107.6, 11.7. 以上数据与文献[13]报道的

Mesaconic acid 一致，故确定该化合物为 Mesaconic acid。

对羟基苯甲酸(*p*-hydroxybenzoic acid)(**7**) $^{1}$H NMR ($CD_3OD$, 600MHz) $\delta$: 7.88 (2H, d, $J$ = 8.4 Hz, H-2, 6), 6.82 (2H, d, $J$ = 8.4 Hz, H-3, 5); $^{13}$C NMR ($CD_3OD$, 150MHz) $\delta$: 170.4 (C-7), 163.5 (C-4), 133.2 (C-2, 6), 123.0 (C-1), 116.2 (C-3, 5). 以上数据与文献[14]报道的对羟基苯甲酸一致，故确定该化合物为对羟基苯甲酸。

circumdatin G(**8**)ESI-MS: *m*/*z* 308 $[M + H]^+$, 330 $[M + Na]^+$, 346 $[M + K]^+$, 637 $[2M + Na]^+$, 653 $[2M + K]^+$, 306 $[M - H]^-$, 613 $[2M - H]^-$, 分子式 $C_{17}H_{13}N_3O_3$; $^{1}$H NMR (DMSO-$d_6$, 600MHz) $\delta$: 8.75 (1H, d, $J$ = 6.0 Hz, NH), 7.77 (1H, dd, $J$ = 7.8, 1.2 Hz, H-4), 7.63 (1H, m, H-5), 7.56 (3H, m, H-6, 7, 12), 7.39 (1H, dd, $J$ = 7.8, 7.8 Hz, H-13), 7.29 (1H, dd, J = 7.8, 1.2 Hz, H-14), 4.27 (1H, m, H-19), 1.57 (3H, d, $J$ = 6.6 Hz, H-20); $^{13}$C NMR (DMSO-$d_6$, 150MHz) $\delta$: 166.7 (C-2), 131.3 (C-3), 128.9 (C-4), 130.6 (C-5), 128.7 (C-6), 128.7 (C-7), 133.2 (C-8), 161.1 (C-10), 121.9 (C-11), 116.6 (C-12), 128.0 (C-13), 119.4 (C-14), 152.9 (C-15), 134.8 (C-16), 154.8 (C-18), 49.7 (C-19), 14.9 (C-20). 以上数据与文献[15]报道的一致，故确定该化合物为 circumdatin G.

(22E,24R)-ergosta-7,22-diene-3β,5α,6β-triol(**9**) ESI-MS: *m*/*z* 453 $[M + Na]^+$, 分子式 $C_{28}H_{46}O_3$; $^{1}$H NMR (Pyridine-$d_5$, 600 MHz) $\delta$: 5.73 (1H, m, H-7), 5.24 (1H, dd, $J$ = 15.0, 7.8 Hz, H-22), 5.18 (1H, dd, $J$ = 15.0, 8.4 Hz, H-23), 4.82 (1H, m, H-3), 4.31 (1H, br s, H-6), 1.53 (3H, s, H-19), 1.05 (3H, d, $J$ = 6.6 Hz, H-27), 0.94 (3H, d, $J$ = 6.6 Hz, H-28), 0.86 (3H, d, $J$ = 6.6 Hz, H-26), 0.86 (3H, d, $J$ = 6.6 Hz, H-21), 0.65 (3H, d, $J$ = 6.6 Hz, H-18); $^{13}$C NMR (Pyridine-$d_5$, 150MHz) $\delta$: 33.0 (C-1), 34.2 (C-2), 68.0 (C-3), 42.4 (C-4), 76.5 (C-5), 74.6 (C-6), 120.9 (C-7), 141.9 (C-8), 44.1 (C-9), 38.4 (C-10), 22.8 (C-11), 40.3 (C-12), 44.1 (C-13), 55.6 (C-14), 23.8 (C-15), 28.8 (C-16), 56.5 (C-17), 12.9 (C-18), 19.2 (C-19), 41.2 (C-20), 20.2 (C-21), 136.6 (C-22), 132.5 (C-23), 43.4 (C-24), 33.7 (C-25), 20.5 (C-26), 21.8 (C-27), 18.2 (C-28). 以上数据与文献[16]报道的一致，故确定该化合物为(22E,24R)-ergosta-7,22-diene-3*β*,5*α*,6*β*-triol.

## 3. 实验部分

### 3.1. 仪器与试剂

Perkin-Elmer 341 旋光仪；Shimadzu UV-160A 紫外光谱仪(Shimadzu Corporation, Japan); Shimadzu FTIR-8400S 红外光谱仪；LTQ Orbitrap XL 质谱仪 (Thermo Scientific, America); Bruker AV-600 核磁共振仪(600 MHz for $^{1}$H and 150 MHz for $^{13}$C) (Bruker Biospin Inc., Germany), tetramethylsilane (TMS)为内标。硅胶 (100-200, 200-300 目, Qingdao Marine Chemistry Ltd., China)； 凝胶 Sephadex LH-20 (20-100 $\mu$, Pharmacia)；薄层硅胶 $GF_{254}$ plates (Yantai Marine Chemical Co., Ltd., China)；制备 HPLC (LUMTECH instrument with a UV detector at 210 nm and using a YMC-Pack $C_{18}$ column) (250 mm × 20 mm inside diameter (I.D.), 5 μm, YMC, Japan)；试剂均为分析纯(北京化工厂)。

### 3.2. 菌种培养

赭曲霉菌株(*Aspergillus ochraceus* 53216)由中科院微生物所提供。采用发酵培养，大米培养基(糙米:水 1:1)，25℃培养 1 个月。

### 3.3. 提取分离

对赭曲霉的大米培养物(8 kg)进行粉碎，用 80%的乙醇溶液加热回流提取 4 次，每次提取时间为 2 小时，合并提取液减压浓缩至无醇味时，有不溶物析出、过滤，滤液转入分液漏斗中，采用等体积乙酸乙酯(含少量甲醇)进行萃取，萃取四次后，合并萃取液，回收溶剂得到乙酸乙酯萃取物(50 g)。对不溶物(40 g)进行硅胶柱层析，石油醚/乙酸乙酯系统(100:0-0:100)洗脱，薄层检测含有毒素的流份合并入乙酸乙酯相，

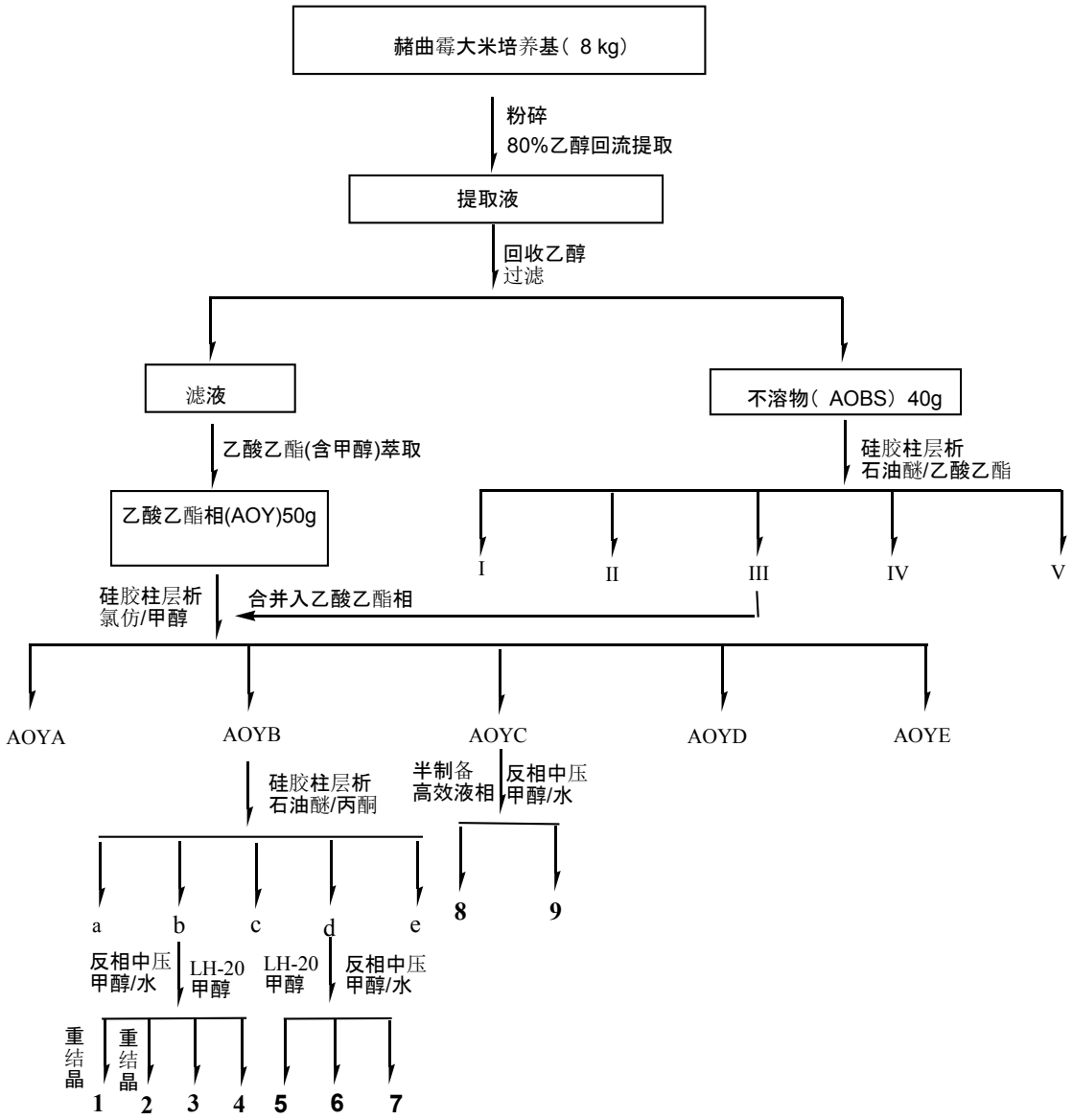


**Figure 2.** Experimental flow chart
**图 2.** 实验流程图

氯仿/甲醇(100:0-0:100)系统洗脱，合并相同流份，得到 AOY A~E5 个部分。AOYB 采用硅胶柱层析，石油醚/丙酮(10:1-0:1)系统洗脱，洗脱前段下来的是一些脂肪状的物质，后面洗脱的流份经薄层检查合并为 a~e 5 个部分。AOYB b 经过反相中压 ODS 柱层析，甲醇/水系统(30%~100%)洗脱，采用凝胶柱层析，甲醇洗脱得到化合物 **3** (5 mg)、**4** (6 mg)、**1** 和 **2** 的粗品。**1** 和 **2** 的粗品经过甲醇重结晶得到化合物 **1** (4 mg)和 **2** (3 mg)。AOYB d 经过反相中压和凝胶柱层析得到化合物 **5** (7 mg)、**6** (10 mg)和 **7** (9 mg)。AOYC 经过反相中压、半制备高效液相得到化合物 **8** (4 mg)和 **9** (5 mg)。流程图见图 2。

## 基金项目

资助信息：湖南省高校产业化培育项目(项目编号：11CY013)；民族药用植物资源研究与利用湖南省重点实验室开放基金项目(项目编号：YYZW2016-11)。

## 参考文献 (References)


[1] El Khoury, A. and Atoui, A. (2010) Ochratoxin A: General Overview and Actual Molecular Status. *Toxins*, **2**, 461-493. https://doi.org/10.3390/toxins2040461

[2] O'Brien, E. and Dietrich, D.R. (2005) Ochratoxin A: The Continuing Enigma. *Critical Reviews in Toxicology*, **35**, 33-60. https://doi.org/10.1080/10408440590905948

[3] Larsen, T.O., Svendsen, A. and Smedsgaard, J. (2001) Biochemical Characterization of Ochratoxin A—Producing Strains of the Genus *Penicillium*. *Applied and Environmental Microbiology*, **67**, 3630-3635. https://doi.org/10.1128/AEM.67.8.3630-3635.2001

[4] Wicklow, D.T., Dowd, P.F., Alfatafta, A.A. and Gloer, J.B. (1996) Ochratoxin A: An Antiinsectan Metabolite from the Sclerotia of *Aspergillus carbonarius* NRRL 369. *Canadian Journal of Microbiology*, **42**, 1100-1103. https://doi.org/10.1139/m96-141

[5] Dais, P., Stefanaki, I., Fragaki, G. and Mikros, E. (2005) Conformational Analysis of Ochratoxin A by NMR Spectroscopy and Computational Molecular Modeling. *The Journal of Physical Chemistry B*, **109**, 16926-16936. https://doi.org/10.1021/jp058035e

[6] Bredenkamp, M.W., Dillen, J.L.M., van Rooyen, P.H. and Steyn, P.S. (1989) Crystal Structures and Conformational Analysis of Ochratoxin A and B: Probing the Chemical Structure Causing Toxicity. *Journal of the Chemical Society, Perkin Transactions*, **2**, 1835-1839. https://doi.org/10.1039/p29890001835

[7] Assante, G., Camarda, L., Locci, R., Merlini, L. and Nasini, G. (1981) Papadopoulos. Isolation and Structure of Red Pigments from *Aspergillus falvus* and Related Species, Grown on a Differential Medium. *Journal of Agricultural and Food Chemistry*, **29**, 785-787. https://doi.org/10.1021/jf00106a023

[8] Ohta, A., Akita, Y., Izumida, A. and Suzuki, I. (1979) Synthesis of *dl*-neohydroxy-aspergillic Acid. *Chemical and Pharmaceutical Bulletin*, **27**, 1316-1320. https://doi.org/10.1248/cpb.27.1316

[9] 魏美燕, 胡谷平, 郑彩娟, 邵长伦, 王长云, 周世宁, 佘志刚, 林永成. 中国南海红树内生真菌 *Microsphaeropsis* sp.二氢异香豆素类化合物研究[J]. 中山大学学报(自然科学版), 2010, 49(2): 68-71.

[10] Devys, M., Barbier, M., Bousquet, J.F. and Kollmann, A. (1994) Isolation of the (–)-(3*R*)-5-hydroxymellein from the Fungus *Septoria nodorum*. *Phytochemistry*, **35**, 825-826.

[11] Sassa, T., Hayakari, S., Ikeda, M. and Miura, Y. (1971) Plant Grown Inhibitions Produced by Fungi. Part I. Isolation and Identification of Penicillic Acid and Dihydropenicillic Acid. *Agricultural and Biological Chemistry*, **35**, 2130-2131.

[12] He, J., Wijeratne, E.M.K., Bashyal, B.P., *et al*. (2004) Cytotoxic and Other Metabolites of *Aspergillus* Inhabiting the Rhizosphere of Snoran Desert Plants. *Journal of Natural Products*, **67**, 1985-1991. https://doi.org/10.1021/np040139d

[13] Collin, P.J. and Sternhell, S. (1966) N.M.R. Spectra of Crotonic, Mesaconic, Citraconic, and *β*, *β*-diluethylacrylic Acids, Their Methyl Esters and Potassium Salts. *Australian Journal of Chemistry*, **19**, 317-320.

[14] 肖美添, 叶静, 洪本博, 咎珂, 屠鹏飞. 白苞蒿化学成分研究[J]. 中国药学杂志, 2011(46): 414-417.

[15] Dai, J.R., Carte, B.K., Sidebottom, P.J., Yew, A.L.S., Ng, S.B., Huang, Y.C. and Butler, M.S. (2001) Circumdatin G, a New Alkaloid from the Fungus *Aspergillus ochraceus*. *Journal of Natural Products*, **64**, 125-126. https://doi.org/10.1021/np000381u

[16] 王兴娜, 杜建厂, 谭仁祥, 刘吉开. 担子菌黄卷缘齿菌的化学成分[J]. 中草药, 2005, 36(8): 1126-1130.