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STRUCTURE STUDIES OF THE METABOLITES OF *PAXILLUS INVOLUTUS*

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Dedicated to the memory of Dr. John Daly, NIH.

Abstract - The chemical components of *Paxillus involutus* were isolated from the freshly collected fruit bodies. Mainly on the basis of the NMR and ESI-MS data, the structures of the unknown pigments were determined as 4-(3,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)-2-(2-pyrrolidon-5-yl)-4-cyclopentene-1,3-dione and (4*Z*)-5-hydroxy-2-(3,4-dihydroxyphenyl)-5-(4-hydroxyphenyl)-2,4-pentadien-4-olide, respectively.

Paxillus involutus (Eng. Brown Roll-rim) is a fair-sized mushroom widely distributed not only in Europe. Following the time of the controversial opinions on the usability of the mushroom for edible purposes,[1] its hemolytic properties were proven in a number of scientific publications, including those based on clinical observations[2] and biochemical tests *in* vitro.[3] The chemical composition of the mushroom is still poorly recognized and there is even no suggestion about the chemical structure, the hemotoxic activity relationship. In our previous paper dealing with the search of *P.involutus* metabolites,[4] in which the structure of involutone, a previously unknown phenolic pigment, was proved, it was pointed out that some artefacts formation occurred due to the enzymatically catalyzed transesterification and transglucosylation reactions with a contribution of the alcohol used in the extractive treatment of the freshly collected fruit bodies.

In the experiments discussed in this paper, the set of pigments occurring in the methanolic extract of lyophilized *P. involutus* was resolved chromatographically on four main compounds, the most abundant involutin[5] and the second one involutone,[4] both of them already described in the literature, and two others (**1** and **2**, respectively) of unknown structures. All of them were found to reveal the antioxidative activity,[6] the highest being observed in the case of involutin. The purified pigment **1** was an optical inactive (in MeOH or MeCN), deep yellow solid, melting with decomposition at 198 ºC. The ^{1}H NMR and ^{13}C NMR

spectra of **1** (^{1}H and ^{13}C assignments were based on COSY, HMQC and HMBC experiments) appeared to be very similar to those of involutone,[4] differing only by the presence of additional signals, which indicated a pyrrolidonyl ring to be a component of the molecule, instead of the Csp^3-OH group. This conclusion was in agreement with the absorption pattern of the IR spectrum.[7] The ESI-MS spectrum exhibited a pseudomolecular ion peak at *m/z* 380 $[M+H]^+$ and a more abundant one at *m/z* 378 $[M-H]^-$, the HR-ESI-MS measurement of which enables us to calculate the molecular formula $C_{21}H_{17}NO_6$ of **1**. The ESI-CID-MS/MS fragmentation spectrum recorded for the anion of *m/z* 378 showed that the pigment molecular ion easily lost a fragment corresponding to the pyrrolidonyl ring and the resulting ion of *m/z* 295 underwent further fragmentation, which was similar to that observed for the cation of *m/z* 295, obtained as a fragmentation ion of involutone, $[M+H-H_2O]^+$. Additionally, the anion of *m/z* 295 easily lost a hydrogen atom, yielding a radical-anion of *m/z* 294, the fragmentation of which followed a similar pathway to that determined for the parent ion of *m/z* 295, the only difference being that the *m/z* values of the corresponding fragmentation ions were each diminished by one mass unit.

On the basis of the presented arguments as well as of ^{1}H, ^{13}C shift correlations, being found as a result of the two dimensional NMR experiments (summarized in Figure 1), the structure of **1** was determined as 4-(3,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)-2-(2-pyrrolidon-5-yl)-4-cyclopentene-1,3-dione.

It is interesting to note that **1** was found to exist in the form of two conformers, quite stable at room temperature, due to the likelihood (as it followed from the Dreiding models inspection) of a strong hydrogen bond formation by the amide proton alternately to one of the two carbonyl oxygens of the cyclopentenedione ring. As a consequence of such a stereochemical inhomogeneity, most of the signals of the proton and carbon NMR spectra were duplicated. The pattern of the duplicated signals (except for H-5') appeared to be temperature dependent. At 25 °C, the ^{1}H NMR spectrum of **1** dissolved in DMSO-d_6, exposed two signals of the proton N-H at 8.07 and 7.94 ppm, which broadened and moved at 60 °C to 7.95 and 7.83 ppm, respectively, while at 80 °C they almost disappeared. Similarly, the resonance pattern of the proton H-5 changed with an increase of temperature. First, a shift of the two singlets was observed from 7.70 and 7.62 ppm (at 25 °C) to 7.58 and 7.52 ppm (at 40-60 °C), respectively, then, at 100 °C, the signals combined into a singlet, which occurred at 7.50 ppm.

The ESI-MS spectrum of the fourth pigment, **2**, displayed the pseudomolecular ion peaks at *m/z* 311 $[M-H]^-$, *m/z* 313 $[M+H]^+$ and *m/z* 335 $[M+Na]^+$, indicating the molecular weight of the compound to be 312. The HR-ESI-MS of the last two cations gave the molecular formula $C_{17}H_{12}O_6$ of **2**. According to the ^{1}H NMR and ^{13}C NMR data, the molecular structure is composed of olefinic and aromatic carbons only and the only carbonyl carbon (the chemical shift of which was found at δ 167.18 ppm) was an element of the lactone function. Additionally, it followed from the proton coupling pattern and chemical shifts that the molecule is composed of 3,4-dihydroxyphenyl and 4-hydroxyphenyl substituents, which are attached

to the carbon chain made of two vinyl groups and lactone carbonyl.[8] On the basis of the presented observations, the two most probable structures for **2** were considered, both of them differing from one another in the localization of the four OH groups in the molecule of 2,5-diphenyl-2,4-pentadien-4-olide skeleton. To solve this problem, the chemical shift values for hydrogens and carbons of these structures were calculated using the ChemDraw Ultra 11.0 program and compared with those determined for the original samples of 3-hydroxy-5-(3,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)-2,4-pentadien-4-olide (trihydroxypulvinone[9]) and of **2**. It followed from the comparison of the calculated results (the most significant differences of chemical shifts[8] were observed in the case of H-5 or 3, H-2', C-2, C-4 and C-5) with the experimental data, that a structure of 5-hydroxy-2-(3,4-dihydroxyphenyl)-5-(4-hydroxyphenyl)-2,4-pentadien-4-olide should be attributed to the pigment marked as **2**. The conclusion was confirmed by ^{1}H, ^{13}C shift correlations, being determined on the basis of the 2D NMR experiments and, additionally, the (4*Z*) configuration of **2** resulted from the through-space interactions (NOESY) of H-3 with H-2' and H-6' only (Figure 1).

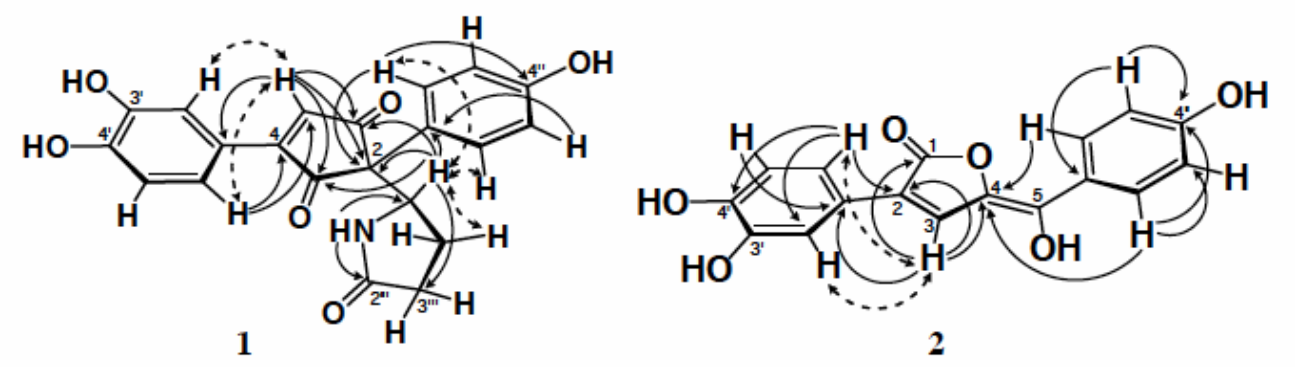

Figure 1. The selected shift correlations in the structure of pigments **1** and **2** resulting from HMBC (⟶) and NOE (⇢) experiments.

The results of searching for the chemical components responsible for the hemolytic properties of *P. involutus,* which were discussed in the initial manuscript of this paper, and were deleted in proof (leading to the isolation, spectral characterization and molecular formula, $C_{28}H_{51}NO_6$, determination of the hemotoxin, which in turn can be hydrolyzed without loss of activity to a mixture of $C_{22}H_{42}O_4$ and $C_6H_8O_3$, accompanied by ammonia elimination), will be published separately.

REFERENCES AND NOTES

1. W. Z. Antkowiak, "*Chemistry and Toxicology of Diverse Classes of Alkaloids: The Chemistry and Toxicology of Mushroom Alkaloids*", ed. by M. S. Blum, Alaken, Inc., Ft. Collins, USA, 1996, pp. 185-300.
2. M. Winkelmann, F. Borchard, W. Stangel, and B. Grabensee, *Dtsch. Med. Wschr.*, 1982, **107**, 1190; M. Winkelmann, W. Stangel, I. Schedel, and B. Grabensee, *Klin. Wochenschr.*, 1986, **64**, 935.
3. S. Habtemariam, *Toxicon*, 1996, **34**, 711.

4. R. Antkowiak, W. Z. Antkowiak, I. Banczyk, and L. Mikolajczyk, *Can. J. Chem.*, 2003, **81**, 118.
5. R. L. Edwards, G. C. Elsworthy, and N. Kale, *J. Chem. Soc. C*, 1967, 405; R. L. Edwards and M. Gill, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1529; R. Feling, K. Polborn, W. Steglich, J. Mühlbacher, and G. Bringmann, *Tetrahedron*, 2001, **57**, 7857, and references cited therein.
6. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. A. Rice-Evans, *Free Radical Biology & Medicine*, 1999, **26**, 1231.
7. Found for **1**: mp 198-199 °C (decomp.); ESI-MS *m/z*: 378 $[M–H]^-$, 757 $[2M–H]^-$, 380 $[M+H]^+$, 402 $[M+Na]^+$, 759 $[2M+H]^+$, 781 $[2M+Na]^+$; HR-ESI-MS: $[M–H]^-$ for $C_{21}H_{16}NO_6$ calcd. 378.09721, found 378.09457; ESI-CID-MS/MS: (-EPI, 378.00) *m/z*: 378, 295, 294; (-EPI, 295.20) *m/z*: 294, 266, 250, 238, 225, 193, 161, 133; ^{1}H NMR (600 MHz, CD_3OD) δ: 2.08 (1H, m, H-4‴), 2.18 (1H, m, H-3‴), 2.30 (2H, m, H-3‴, H-4‴), 4.50, 4.52 (1H, 2dd, H-5‴), 6.78, 6.79 (2H, 2d, *J* = 8.8, H-3″, H-5″), 6.86, 6.88 (1H, 2d, *J* = 8.3, H-5′), 7.27, 7.28 (2H, 2d, *J* = 8.8, H-2″, H-6″), 7.44, 7.47 (1H, 2s, H-5), 7.55, 7.56 (1H, 2dd, *J* = 8.3, 2.2, H-6′), 7.58, 7.59 (1H, 2d, *J* = 2.1, H-2′). For a solution of **1** in DMSO-d_6 two additional signals at 7.94 and 8.07 of NH proton were observed. ^{13}C NMR (150 MHz, CD_3OD) δ: 22.73 (C-4‴), 30.60 (C-3‴), 59.58, 59.62 (C-5‴), 62.25, 62.53 (C-2), 116.71 (C-5′), 116.79, 116.81 (C-3″, C-5″), 117.30, 117.36 (C-2′), 121.88, 122.06 (C-1′), 124.04, 124.19 (C-6′), 125.78, 125.84 (C-1″), 129.58, 129.61 (C-2″, C-6″), 139.78, 139.85 (C-5), 146.81, 146.87 (C-3′), 151.14, 151.17 (C-4′), 158.22, 158.38 (C-4), 158.75, 158.77 (C-4″), 181.76, 181.86 (C-2‴), 202.62, 203.54, 204.06, 205.14 (C-1, C-3); IR (KBr) cm^{-1}: 3241, 2955, 2930, 1732, 1683, 1609, 1593, 1573, 1512, 1443, 1383, 1245, 1181, 1119, 870, 823.
8. Found for **2**: ESI-MS *m/z*: 311 $[M–H]^-$, 313 $[M+H]^+$, 335 $[M+Na]^+$; HR-ESI-MS: $[M+Na]^+$ for $C_{17}H_{12}O_6Na$ calcd. 335.05261, found 335.05149; ESI-CID-MS/MS: (+MS2, 313.00) *m/z*: (35V) 313, 295, 267, 249, 239, 191, 163, 151, ^{1}H NMR (600 MHz, CD_3OD) δ: 7.54 (1H, s, H-3), 7.21 (2H, d, *J* = 8.6, H-2″, H-6″), 6.88 (1H, d, *J* = 2.0, H-2′), 6.85 (2H, d, *J* = 8.6, H-3″, H-5″), 6.81 (1H, d, *J* = 8.2, H-5′), 6.76 (1H, dd, *J* = 8.1, 2.0, H-6′); ^{13}C NMR (150 MHz, CD_3OD) δ: 165.89 (C-1), 156.92 (C-4″), 147.92 (C-3), 145.46 (C-4′), 144.92 (C-3′), 131.81 (C-2″, C-6″), 128.17 (C-5), 122.66 (C-1′), 122.13 (C-1″), 120.82 (C-6′), 119.25 (C-2), 116.45 (C-2′), 115.07 (C-3″, C-5″), 114.99 (C-5′), 105.84 (C-4); The chemical shift values of H-5 or 3, H-2′, C-2, C-3, C-4 and C-5 calculated for the structures of trihydroxypulvinone: 6.06, 7.17, 94.5, 166.5, 141.0, 107.8, and of the second isomer, which was found to be identical to **2**: 7.54, 6.72, 133.4, 135.7, 109.0, 133.2, respectively; IR (KBr) cm^{-1}: 3279 (br.), 1683, 1608, 1515, 1445, 1384, 1256, 1175, 1117, 1042, 836, 817.
9. R. Ramage, G. J. Griffiths, F. E. Shutt, and J. N. A. Sweeney, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1539.