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Tautomerism between exomethylene type physalins and oxymethylene-bridged physalins

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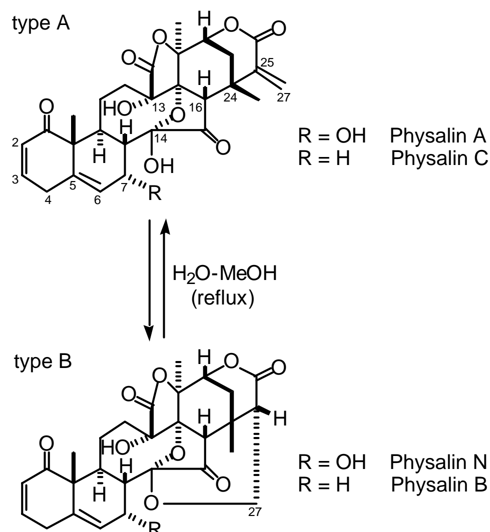
Physalins are steroidal constituents of *Physalis* [1–9], *Witheringia* [10], *Deprea* [11] and *Saracha* [12] plants (Solanaceae) which have cytotoxic and antitumor activities [10, 13, 14]. Since the isolations of physalins A and B from *Physalis alkekengi* var. *francheti* (Japanese name; Hozuki) [1], more than 20 physalins were isolated. Except physalin P with a rearranged neophysalin skeleton [2] and physalins R and S containing an additional C–C linkage [3], physalins commonly possess a 16,24-cyclo-13,14-secoergostane framework. According to the presence or absence of C(14)–O–C(27) acetalic linkage, physalins are classified to types B or A. Physalins A [1], C [4], L [5], M [6] and O [7] and (25*S*)-25,27-dihydrophysalin C [10] belong to type A, while the other physalins including physalins B [1] and N [7] are type B. Among the physalins of type A, physalins A and C possess an exocylic double bond C(25)=C(27), while in physalins L, M and O and the dihydrophysalin C the C(27) atom constitutes a secondary methyl group with (*S*)-configuration. In this communication we will describe tautomerism between the physalins of type A possessing an C(27) exomethylene group and the corresponding physalins of type B as shown in the Scheme. This interconversion enabled preparation of physalin derivatives containing a terminal methylene group and also their dihydro derivatives which could be submitted to biological analysis.

Scheme



In our attempt to hydrogenate physalin N [7] over palladium-carbon catalyst using tetrahydrofuran as a solvent, 2,3-dihydrophysalin A was isolated as a by-product (27%) in addition to the desired 2,3-dihydrophysalin N (50%). Since the former product contains a C(25)=C(27) double bond which could be easily hydrogenated, the physalin A derivative was considered to be formed from the expected dihydrophysalin N after the catalytic hydrogenation procedure. Extensive studies using a number of physalins revealed that in certain conditions the physalins of type B are in equilibrium with the corresponding physalins of type A possessing an exocyclic methylene group. Refluxing the suspension of a physalin in 50% aqueous methanol was found to be an efficient method for obtaining a tautomeric mixture of physalins of type A and type B.

Although physalin C was reported in 1970 [4], isolation of a significant amount of physalin C could not be reproduced. However, by the use of this equilibration procedure physalin C was obtained in 31% yield from abundantly available physalin B and was subjected to cytotoxic assay [14]. When physalin D (5α,6β-dihydroxy-5,6-dihydrophysalin B) [8] was subjected to the tautomeric equilibration 5α,6β-dihydroxy-5,6-dihydrophysalin C was obtained in 23% yield. Physalin F (5β,6β-epoxy-5,6-dihydrophysalin B) [9] similarly yielded the expected 5β,6β-epoxy-5,6-dihydrophysalin C in 23% yield along with the epoxide-opened 5α,6β-dihydroxy compounds. Physalin P possessing a neophysalin structure was recovered unchanged under the same conditions.

The tautomeric equilibration was also applied to the preparation of physalin derivatives with a C(27)-secondary methyl group. Physalins of type B were subjected to catalytic hydrogenation in the presence of triethylamine in methanol or methanol-tetrahydrofuran mixture at room temperature. Physalin B in methanol yielded (25*S*)-2,3,25,27-tetrahydrophysalin C (92%) and (25*S*)-2,3,5,6,25,27-hexahydrophysalin C (8%). Physalin N similarly yielded (25*S*)-2,3,25,27-tetrahydrophysalin A in high yield (94%), but reaction in tetrahydrofuran without triethylamine afforded 4 : 1 mixture of (25*S*)- and (25*R*)-2,3,25,27-tetrahydrophysalin A in 77% yield.

Experimental

1. Materials

Physalins were isolated from epigeal parts of *P. alkekengi* var. *francheti* as described in the literature [1–7].

2. Methods

Typical reaction procedures are as follows.

Physalin C from physalin B: A suspension of physalin B (211 mg) in MeOH-H_2O (1 : 1, 50 ml) was refluxed for 5 h to give a clear solution. Solvent was evaporated and the residue was chromatographed over silica gel (Silica Gel 60, 230–400 mesh, Merck) using $CHCl_3$-acetone as eluent to afford recovered physalin B (105 mg) and physalin C (66 mg).

(25*S*)-2,3,25,27-Tetrahydrophysalin C from physalin B: Et_3N (0.2 ml) was added to the solution of physalin B (309 mg) in MeOH-THF (3 : 1, 40 ml), which was subjected to catalytic hydrogenation over 5% Pd-carbon (155 mg) with atmospheric H_2 at room temperature for 2 h. Usual workup and silica gel column chromatography using $CHCl_3$-MeOH as eluent afforded tetrahydrophysalin C [1] (286 mg) and the extensively reduced (25*S*)-2,3,5,6,25,27-hexahydrophysalin C [1] (27 mg).

References

1 Matsuura, T., Kawai, M., Nakashima, R., Butsugan, Y.: J. Chem. Soc. (C), 664 (1970)
2 Kawai, M.; Matsumoto, A.; Makino, B.; Mori, H.; Ogura, T.; Butsugan, Y.; Ogawa, K.; Hayashi, M.: Bull. Chem. Soc. Jpn. **66**, 1299 (1993)
3 Makino, B.; Kawai, M.; Kito, K., Yamamura, H.; Butsugan, Y.: Tetrahedron **51**, 12529 (1995)
4 Kawai, M.; Matsuura, T.: Tetrahedron **26**, 1743 (1970)
5 Kawai, M.; Matsuura, T.; Kyuno, S.; Matsuki, H.; Takenaka, M.; Katsuoka, T.; Butsugan, Y.; Saito, K.: Phytochemistry **26**, 3313 (1987)
6 Kawai, M.; Ogura, T.; Nakanishi, M.; Matsuura, T.; Butsugan, Y.; Mori, Y.; Harada, K.; Suzuki, M.: Bull. Chem. Soc. Jpn. **61**, 2696 (1988)
7 Kawai, M.; Ogura, T.; Makino, B., Matsumoto, A.; Yamamura, H.; Butsugan, Y.; Hayashi, M.: Phytochemistry **31**, 4299 (1992)
8 Row, L. R.; Sarma, N. S.; Reddy, K. S.; Matsuura, T.; Nakashima, R.: Phytochemistry **17**, 1647 (1978)
9 Row, L. R.; Reddy, K. S.; Sarma, N. S.; Matsuura, T.; Nakashima, R.: Phytochemistry **19**, 1175 (1980)
10 Antoun, M. D.; Abramson, D.; Tyson, R. L.; Chang, C.-J.; McLaughlin, J. L.; Peck, G.; Cassady, J. M.: J. Nat. Prod. **44**, 579 (1981)
11 Luis, J. G.; Echeverri, F.; Quiñones, W.; González, A. G.; Torres, F.; Cardona, G.; Archbold, R.; Perales, A.: Tetrahedron **50**, 1217 (1994)
12 Ripperger, H.; Kamperdick, C.: Pharmazie **53**, 144 (1998)
13 Makino, B.; Kawai, M.; Ogura, T.; Nakanishi, M.; Yamamura, H.; Butsugan, Y.: J. Nat. Prod. **58**, 1668 (1995)
14 Kawai, M.; Makino, B;.Yamamura, H.; Araki, S.; Butsugan, Y.; Ohya, J.: Pharmazie, in print

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Potassium titanyloxalate as analytical reagent for micro-quantitative determination of quercetin

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Quercetin ($C_{15}H_{10}O_7$; 3,3′,4′,5,7-pentaxydroxyflavone) is the aglycon of the flavonol type for a great number of different glycosides; one of them is rutin which has therapeutic action and is mostly applied as a drug for curing blood vessel diseases.

Quercetin as well as rutin has been determinated spectrophotometrically *via* a complexing reaction with many metal ions [1–6]. In our previous paper [7], we investigated the complexation reaction of rutin with the potassium-titanyloxalate, $K_2[TiO(C_2O_4)_2]$ (PTOx) which significantly lowered the detection limit for rutin determination.

Thus, the aim of the present study was to investigate the titanyloxalato-quercetin complex in order to use this particular reaction of complexation to improve detection limits for the determination of quercetin.

PTOx and quercetin form a complex of distinctive yellow-orange color. The intensity and hue of the color are strongly dependent on the pH, the concentration of the reactants, the ionic strength and temperature. The complex formation was investigated in a wide pH range from 3.6 to 10.0 (Fig. 1).

In the pH range from 3.6 to 5.6, the complex spectra shows the absorption maximum at $\lambda = 420$ nm. At higher pH values, the absorption maxima are batochromly shifted. Since the observed batochromic shift may be caused either by formation of complexes with different stoichiometric composition or by dissociation of the already existing complex, the composition of complex was determined at several pH values: in the pH range without any shifts (pH = 3.6 and pH = 4.3) and in the pH range where the shift is of about 10 nm (pH = 7.2). Examining on higher pH values is useless, since hydrolysis of the titaniloxalate ion implies decreasing of liberate metal ion.

The stoichiometric composition of the complex was investigated by the method of continual variations of equimolar solutions [8] and by the molar ratio method [9]. According to the former method, mixed solutions of PTOx and

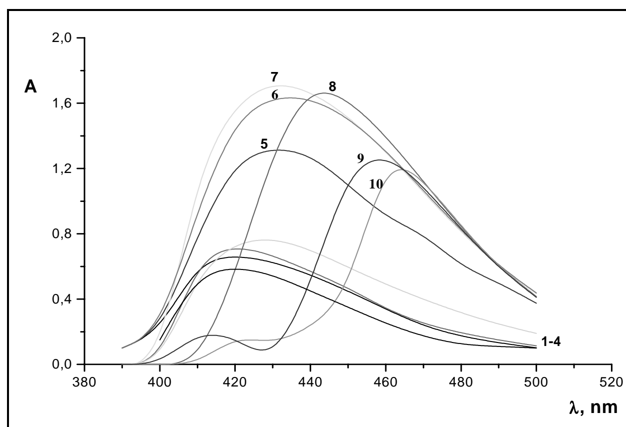

Fig. 1: Absorption spectra of the complex of quercetin, $c_{Querc} = 5.0 \times 10^{-4}$ M and potassium titanyloxalate, $c_{PTOx} = 2.5 \times 10^{-5}$ M, obtained for different values of pH (curves 1–10): 3.6 (1), 4.3 (2), 5.0 (3), 5.6 (4), 6.4 (5), 6.9 (6), 7.2 (7), 8.2 (8), 9.2 (9) and 10.0 (10). The ionic strength is $I = 7.5 \times 10^{-5}$ M, and the temperature T = 296 K. Blank for all cases is quercetin in the same concentration, pH, ionic strength and temperature as in the mixture.