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CASEIN KINASE II INHIBITORS ISOLATED FROM TWO BRAZILIAN PLANTS *HYMENAEA PARVIFOLIA* AND *WULFFIA BACCATA*

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Abstract: Two dihydroflavonol rhamnosides (**1** and **2**) isolated from the bark of *Hymenaea parvifolia* and two pentacyclic triterpenoids (**3** and **6**) obtained from the leaves of *Wulffia baccata* have been found to exhibit inhibitory effects of casein kinase II (CK-II) dose-dependently, suggesting that at higher doses more than 10 μM, these four compounds may act as potent CK-II suppressors of the CK-II-mediated activation of 60S acidic ribosomal P proteins in vitro.

Introduction

Recent reports have demonstrated that casein kinase II (CK-II), a scond messenger-independent nuclear serine/threonine protein kinase, is characterized as one of protein kinases responsible for positive and negative regulations by specific phosphorylation of several DNA-binding transcriptional factors,[1] and therefore CK-II plays an important role as a controller and/or a mediator of various cell functions.[2] Recently several novel functions of CK-II inhibitors and activators have been disclosed, *e.g.*, as a cellular mediator responsible for promoting HIV-1 replication in virus-infected cells.[3] During our studies on search for bioactive natural products, we recently investigated two neotropical plants *Hymenaea parvifolia* and *Wulffia baccata* collected in Brazil. Among the chemical constituents of these plants, two dihydroflavonol rhamnosides, neoastilbin (**1**) and astilbin (**2**), and two pentacyclic triterpenoids, lantic acid (**3**) and camaric acid (**6**), were revealed to exhibit inhibitory effects of casein kinase II (CK-II) in a manner similar to catechin.

Chemistry

The bark of the neotropical leguminous plant, *Hymenaea parvifolia* L. (Caesalpinioideae), is called "Jutai" in Brazil, and used as an astringent or an analgesic. From a trunk resin sample of this plant, a diterpene acid (enantio-13-epilabdanolic acid) has been isolated as the major resin acid.[4] The bark of *Hymenaea parvifolia* was treated with EtOH. The EtOAc-soluble fraction of the EtOH extract was subjected to repeated

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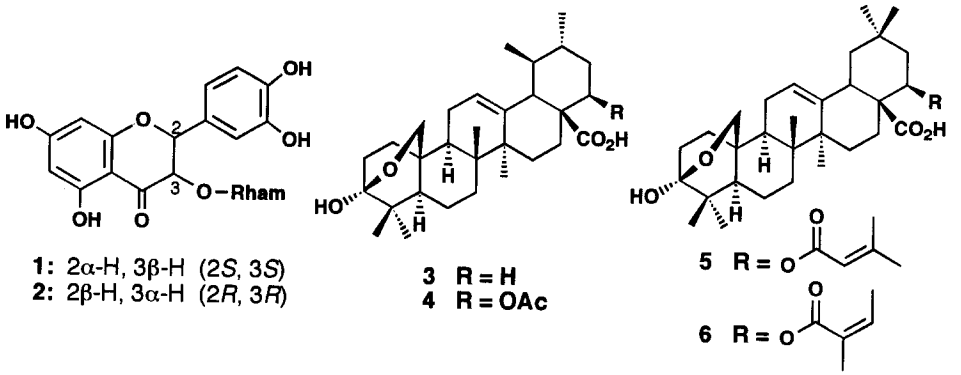

chromatographies on silica gel and Sephadex LH-20, followed by purification with HPLC on ODS to give two dihydroflavonol rhamnosides, neoastilbin (**1**) and astilbin (**2**), which were identified on the basis of comparison of their spectral data with those in the literature.[5,6] These dihydroflavonol rhamnosides were previously isolated from plant sources such as leaves of *Engelhardtia chrysolepis* (Juglandaceae), a Chinese sweet tea,[5] or from a fern *Sphaerostephanos arbuscula*.[6] Isolation of compounds **1** and **2** from the bark of *Hymenaea parvifolia* has not been reported before. On the other hand, *Wulffia baccata* O. Kuntze (Compositae) is called "Cambará" in Brazil, and studies on the chemical constituents of this plant have never been reported so far. The leaves of *Wulffia baccata* was treated with EtOH, and the combined EtOAc- and *n*-BuOH-soluble fractions of the EtOH extract were subjected to fractionation by repeated chromatography on silica gel, Sephadex LH-20, and HPLC on ODS to give triterpenoids **3** ~ **6**. The structures of these triterpenoids were identified as lantic acid (**3**), camarinic acid (**4**), lantanilic acid (**5**), and camaric acid (**6**), respectively, on the basis of comparison of their spectral data with those reported in the literature.[7] These triterpenoids were previously isolated from *Lantanacamara* (Verbenaceae).[7] This is the first chemical study on the constituents in the leaves of this plant *Wulffia baccata*.[8]

Materials and Methods

Plant Materials. Both plants, *Hymenaea parvifolia* and *Wulffia baccata*, were collected in January 1995 at Belém, Pará, Brazil, and identified by Dr. J. I. Gomes by comparison with voucher specimens (IAN 144.541 and IAN 174.126, respectively) deposited at the Harbarium of Centro de Pesquisa Agroflorestal da Amazônia oriental.

Extraction and Isolation. (i) The bark of *Hymenaea parvifolia* (50 g, dry weight) was extracted with EtOH (300 mL x 3). The EtOH extract (2.8 g) was partitioned between EtOAc (120 mL x 3) and H_2O, and the aqueous phase was further extracted with *n*-BuOH (120 mL x 3). A part of EtOAc-soluble fraction (0.21 g) was subjected to silica gel column chromatography (1.3 x 12 cm) eluted with MeOH/$CHCl_3$ (0% to 100%). The fraction (69 mg) eluted with 12 % MeOH/$CHCl_3$ was further separated by gel filtration on Sephadex LH-20 (1.5 x 70 cm; MeOH) to give a fraction (63 mg) containing a Fast Red B positive spot on TLC plates. This fraction was then purified with HPLC (PEGASIL ODS, 20 x 250 mm; eluent, 40% MeOH) to give give neoastilbin (**1**, 11.1 mg) and astilbin (**2**, 13.0 mg). (ii) The leaves of *Wulffia baccata* (50 g, dry weight) were extracted with EtOH (300 mL x 3). The EtOH extract (1.7 g) was partitioned between EtOAc (140 mL x 3)

and H_2O, and the aqueous phase was further extracted with *n*-BuOH (140 mL x 3). The combined EtOAc- and *n*-BuOH-soluble fractions (0.3 g) was subjected to silica gel column chromatography (1.2 x 16 cm) eluted with MeOH/$CHCl_3$ (0% to 100%). The fraction (93.4 mg) eluted with 2-5 % MeOH/$CHCl_3$ was further separated by gel filtration on Sephadex LH-20 (1.5 x 66 cm; MeOH) to give lantic acid (**3**, 35.6 mg) together with a fraction (45.2 mg) containing a mixture of triterpenoids. This fraction was then separated by a silica gel column chromatography (0.8 x 12 cm) eluted with AcOEt/hexane (1:2) to afford camarinic acid (**4**, 12.2 mg) along with a fraction (31.5 mg) of a triterpenoid mixture, which was finally purified with HPLC (PEGASIL ODS, 20 x 250 mm; eluent, 95% MeOH) to give lantanilic acid (**5**, 4.5 mg) and camaric acid (**6**, 8.2 mg), together with lantic acid (**3**, 12.1 mg).

Purification of 60S Acidic Ribosomal P Proteins Associated with CK-II. The 60S acidic ribosomal P proteins [P0 (p35), P1 (p15) and P2 (p13)] were extracted from young tissues of young bamboo shoots, and partially purified by means of successive DEAE-cellulose and Heparin-sepharose column chromatographies, and gel filtration on a Superdex 200pg HPLC column, as previously reported.[9] The purified 60S acidic ribosomal P proteins (p35, p15 and p13) consisted of a hetero-complex associated with CK-II. This hetero-complex provides convenient materials for an in vitro assay of CK-II activity, since CK-II in the complex effectively phosphorylates these three proteins (p35, p15 and p13) by incubation with either [γ-^{32}P]ATP or [γ-^{32}P]GTP in the presence of a suitable CK-II acitivator, such as poly-Arg and protamine, as reported previously.[9]

Assay for CK-II and Autoradiography. To measure CK-II activity (phosphorylation of 60S ribosomal P proteins), the standard reaction mixtures (100 μL) comprised 40 mM Tris-HCl (pH 7.6), 2 mM dithiothreitol (DTT), 3 mM Mn^{2+}, 5 μg of purified 60S acidic ribosomal P proteins associated with CK-II, poly-Arg (5 μg, a CK-II activator) and 20 μM [γ-^{32}P]ATP (1,000 cpm/pmol). After incubation for the indicated periods at 10

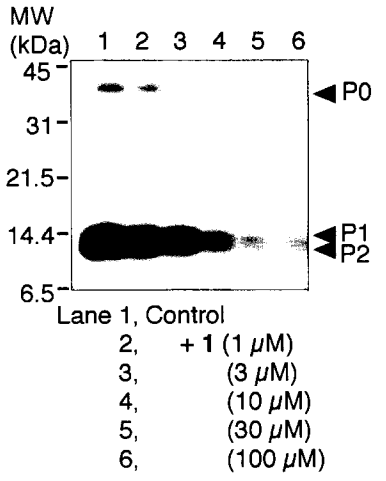

Fig. 1. The inhibitory kinetics of CK-II activity (phosphorylation of 60S acidic ribosomal P proteins) by compound **1**. ^{32}P-labelled p35, p15, and p13 were detected by SDS-PAGE followed by autoradiography.

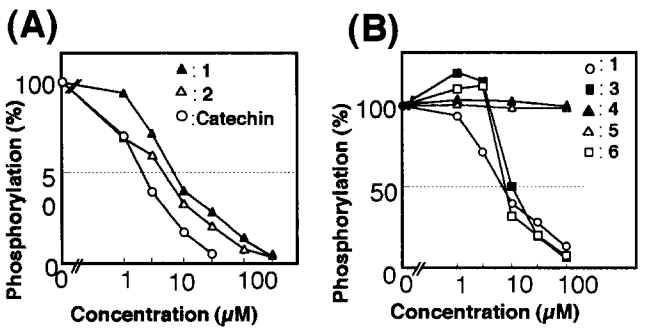

Fig. 2. The inhibitory effects on CK-II activity of (A) compounds **1** and **2**, and catechin, and (B) compounds **1** and **3** ~ **6** in a dose-dependent manner. 100% represents the CK-II-mediated phosphorylation of p35, p15 and p13 determined without these compounds.

°C, the ^{32}P-labeled polypeptides (p35, p15 and p13) were detected by SDS-PAGE followed by autoradiography, as reported previously.[2,3,9]

Results and Discussion

To detect phosphorylation of 60S acidic ribosomal P proteins (p35, p15 and p13) by CK-II purified from young bamboo shoots, the purified fraction (Superdex fraction) was directly incubated with 20 μM [γ-^{32}P]ATP (1,000 cpm/pmol) in the presence of poly-Arg (a CK-II activator). Under the experimental conditions, three distinct proteins (p35, p15 and p13) were detected as phosphorylated polypeptides (Fig. 1). These results show that CK-II is a kinase responsible for specific phosphorylation of these 60S acidic ribosomal P proteins (p35, p15 and p13) in the presence of poly-Arg in vitro.

The inhibitory effect of compounds **1** and **2** isolated from *H. parvifolia* on CK-II activity was determined in vitro as a comparative control experiment. Compounds **1** and **2** inhibited CK-II activity in a dose-dependent manner, as shown for catechin (Fig. 2A), and the 50% inhibition values (ID_{50}) of **1** and **2** were found to be *ca.* 7 and 9 μM, respectively. No significant difference of the inhibitory effects on CK-II-mediated phosphorylation between three proteins (p35, p15 and p13) was detected. A similar inhibitory effect of compound **1** was observed when other CK-II substrates, such as Hsp-90, phosvitin and casein, were used instead of 60S acidic ribosomal P proteins as a phosphate acceptor for purified CK-II in vitro (data not shown). Under the same experimental conditions, two compounds **3** and **6** isolated from *Wulffia baccata* also inhibited CK-II activity in a dose-dependent manner, as shown for **1** (Fig. 2B). However, no effects of compounds **4** and **5** on CK-II were detected. These results suggest that, at higher doses more than 10 μM, these plant compounds (**1**, **2**, **3**, and **6**) act as potent CK-II inhibitors of 60S acidic ribosomal P proteins in vitro.

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