An Enzyme-Mimetic Chemical Conversion and Biogenetic Outline for Chemical Marker Pterocarponoid Oxygen Heterocycles from Kudzu Vine

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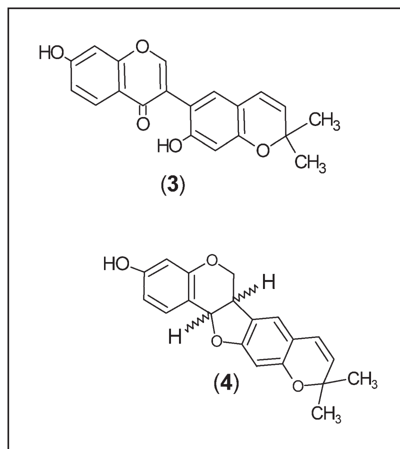

An enzymatic chemical conversion of isoflavone and chemical marker oxygen-heterocycles pterocarponoids are carried out in an analogy to major subcellular enzymatic conversions involved in the natural biogenetic pathway and microbial interaction steps for oxygen-proliferated products obtained from bioactive fractions of widely occurring Kudzu plant, *Pueraria tuberosa*. An outline for biogenesis of major pterocarponoid constituents from principal biogenetic precursor, deoxytuberosin, is also proposed for *P tuberosa*.

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INTRODUCTION

Isoflavonoids, an oxygen-bearing plant heterocycles, are significant subclass of phytochemically abundant flavonoid natural products and form major and vital constituents of Soya and Kudzu plants derived phytomedicines [1–5]. The plants and their parts have been found to possess wide spectrum of biological activities including protection against stress-induced myocardial ischemia, nootropic activity, helpful in ameliorating conditions of migraine and deafness, antioxidant properties, dose-dependent hepatotoxicity, antimicrobial properties [6–10], uses in estrogen replacement therapies, an emergency food and as a food supplement [11–15]. The isoflavonoids play significant roles in plant–microbe interactions at biology-environment interface [16,17] and are considered to be involved in signal transductions [18–20], contributions to root-nodules formation in leguminous plants [21–23] and their functions as plant antibiotics.

The biosynthesis of isoflavonoids has been studied in depth including the identification of genes [24–29], isolation and characterization of enzymes, and tracing of the biochemical pathways of conversions [30–32]. The isoflavonoid biogenesis is now firmly established and links between isoflavones and various pterocarponoid categories have been investigated at molecular details leading to identification of enzymes involved [33–37]. The major enzymes are membrane-bound, including 2-hydroxyisoflavanone synthase responsible for the formation of isoflavone nucleus, pterocarpan synthase, pterocarpan-6a-synthase (P6aH), pterocarpan-3-O-methyltransferase (P3OM), prenyl transferase and prenyl cyclase, associated with different steps of isoflavone, and pterocarponoid biogenesis [38,39].

RESULTS AND DISCUSSION

During the search for postcoital anti-implantation active phytoconstituent(s) from *Pueraria tuberosa* vine, the 95% aqueous ethanolic extracts of fresh tubers exhibiting both estrogenic and anti-estrogenic hormonal bioactivity in mammalian test models [40], a notable structural correlation among isolated isoflavone and

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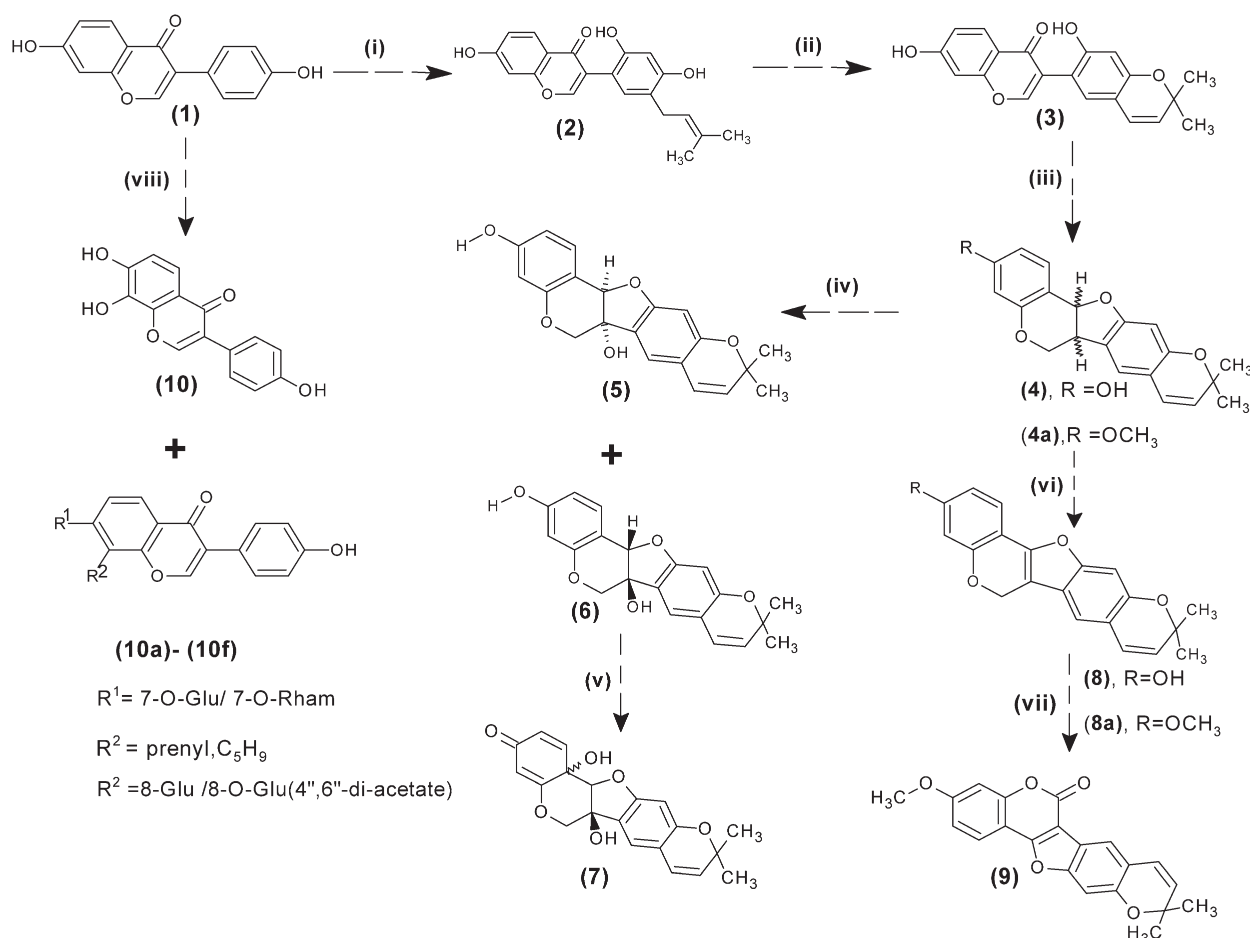
Scheme 1. Biogenetic outline.



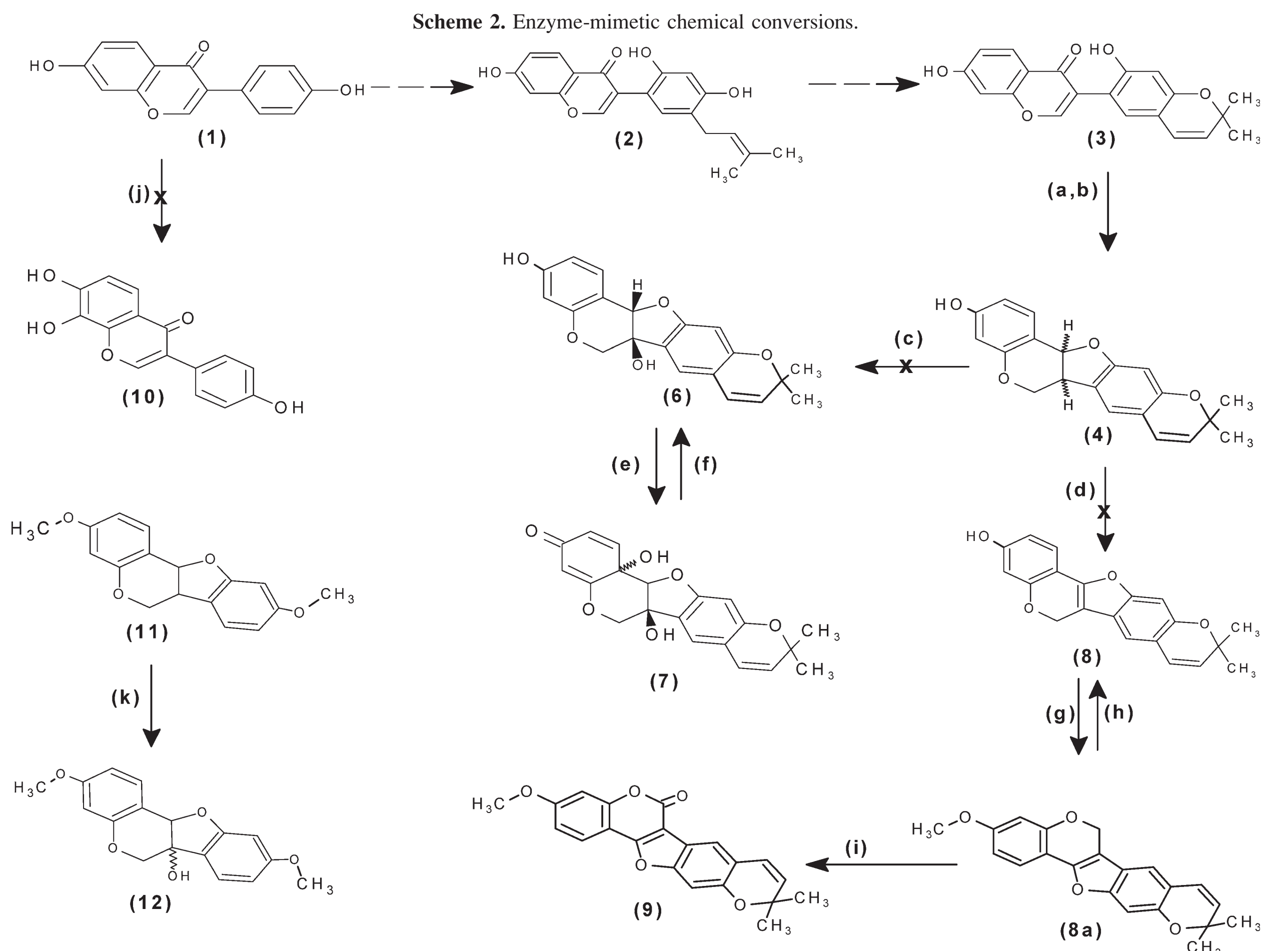
Biogenetic Steps: (i) Hydroxylation & prenylation, (ii) prenyl cyclisation, (iii) stereo-specific conjugated ketone reduction & ring formation-cyclisation, (iv) 6a-oxidation, (v) phenol-quinonol conversion, (vi) 6a-11a reduction, (vii) allylic oxidation, (viii) hydroxylation/prenylation/glycosylation., Glu=Beta-D-Glucosyl, Rham= Rhamnosyl sugar groups.

pterocarponoids was observed. Based on oxidation states and oxygen atom's gradual proliferation in major isoflavonoid and pterocarponoid constituents in an increasing order of molecular weight, a sequence of biogenetic conversions is proposed (Scheme 1). A series of biochemical transformations are envisioned starting from 4′,7-dihydroxyisoflavone (daidzein) (**1**) to neobavaisoflavone (**2**), chromenoisoflavone, puerarone (**3**) and pterocarpan derivative, deoxytuberosin (**4**). The isolation of these products from the plant extracts represented the outcome of initial steps of chromeno-isoflavone biogenesis in the plant. The formation of pterocarpan, deoxytuberosin (**4**) as a sequel of advancing biogenesis from daidzein (**1**) to neobavaisoflavone (**2**) formed by C-3′ carbon prenylation, and C-6′ carbon's hydroxylation followed by C-3′ prenyl group cyclization to yield dimethylchromeno-isoflavone, puerarone (**3**), all established the front-line of biogenetic sequence defining the connection between isoflavone and pterocarponoid oxygen heterocycles as well as suggesting a downstream possible route of biogenetic events. The analogous stereospecific bioreduction [41,42] of intermediate isoflavone, puerarone (**3**), cyclization of the resulting isoflavan-4-ol to afford racemic pterocarpan, (±) deoxytuberosin (**4**), and transformation of this principal pterocarponoid biogenetic precursor, (±) deoxytuberosin (**4**) to other chromeno-pterocarpanoids by incorporating molecular oxygen into the racemic pterocarpan (**4**) to predictably produce pterocarpan-6a-ol(s) (**5**) and (**6**), namely isotuberosin [(−) tuberosin (**5**)] and tuberosin [(+) tuberosin (**6**)] is in line with other studies reported on leguminous plants involving different and nearly similar structural templates of pterocarponoid biosynthetic patterns including initial proposals [41–44] for *P. tuberosa.*

Furthermore, in the later events, a possible monooxygenase mechanism involving pterocarpan-6a-ol, tuberosin (**6**) to produce pterocarpanone, 1a-hydroxytuberosone (**7**) again substantiated the biogenetic proposal, Scheme 1. The dehydrogenation of deoxytuberosin (**4**) to yield anhydrotuberosin (**8**), which by late biogenetic O-methylation produces 3-O-methylanhydrotuberosin (**8a**) and the biochemical allylic oxidation of the C-6-methylene function in 3-O-methylanhydrotuberosin (**8a**) resulting in the production of pterocarpone/coumestan nucleus, tuberostan (**9**), provided the pterocarpene and coumestan class of pterocarponoid compounds respectively in the progressing biogenetic line. The isolation of newly found isotuberosin (**5**) from this plant and tuberosin (**6**), both from the bioactive chloroform extracts of *Pueraria tuberosa* was conclusive of the involvement of stereospecific pterocarpan precursor deoxytuberosin (**4**), isolated here as a racemic mixture, to produce these pterocarpan-6a-ol(s) (**5**) and (**6**), and thus, unequivocally establishing the involvement of stereo-specific reduction of isoflavone transformation in this plant also. The pterocarpan, deoxytuberosin (**4**), and pterocarpan-6a-ol isotuberosin (**5**) were isolated in trace amounts from the plant when compared with pterocarpan-6a-ol, tuberosin (**6**), 1a-hydroxytuberosone (**7**), anhydrotuberosin (**8**), and 3-O-methylanhydrotuberosin (**8a**). The insignificant concentrations of product (**4**) strongly indicated the possibility of its involvement as an intermediate template-pool of molecule for advancing the biogenesis in producing new pterocarponoid compounds. The probable participation of products (**4**) and (**5**) as *in situ* antimicrobial agent and (**6**) being tested and a known antimicrobial agent against certain plant-pathogens, indicated the possibilities of stimulus-responsive plant biogenesis of secondary metabolites. The highly oxygenated pterocarpanone subclass of pterocarponoids in comparison with pterocarpan-6a-ol (constituent oxygen ratio, 27.09:23.64, respectively), also classified as ''stress metabolites,'' are generally considered to be biogenetically derived from pterocarpan-6a-ol by oxygen proliferation owing to its participation in the detoxification process in response to external stimuli (*e.g*., microbes, wounds, etc.) in the plants' environment [45]. The generation of 1a-hydroxytuberosone (**7**) from tuberosin (**6**) indicated likewise but, the generation of these classes of pterocarponoids is also encountered in other leguminous plants, the possibility of them being generated on a genetically planned biogenetic lines as part of leguminous plant defense seems more plausible as their occurrence can not be considered specific to some leguminous plants. It is also suggested that deoxytuberosin (**4**) is the principal phytoalexin (plant antibiotic) of *P. tuberosa* and immediate biogenetic precursor of pterocarpan-6a-ol (**5**) and (**6**), wherein both probably are a product of milder external stimuli and hereby suggesting a response-related biogenesis. The product (**6**)—a tested and known antimicrobial active constituent, is produced as a milder-active phytoalexin, whereas the pterocarpanone, 1a-hydroxytuberosone (**7**) is the final product of the stimulus generated biochemical response's detoxification process common to a variety of leguminous plants. A study showing weak antifungal activity of tuberosin (**6**) and no antifungal activity in 1a-hydroxytuberosone (**7**) also indicated likewise for its plant defense roles and the bioecological outcome. Again, the term ''stress metabolite'' is also applicable to pterocarpone or coumestan oxygen heterocylces, tuberostan (**9**), which is a product of allylic oxidation of 3-O-methylanhydrotuberosin (**8a**) in response to certain stimulus [46,47], whereas pterocarpene, anhydrotuberosin (**8**), and 3-O-methylanhydrotuberosin (**8a**) are envisioned to be produced from pterocarpan deoxytuberosin (**4**) and/or 3-O-methyldeoxytuberosin (**4a**), Scheme 1. The anhydrotuberosin (**8**) and 3-O-methylanhydrotuberosin (**8a**) represented the pterocarpene class, whereas tuberostan (**9**) and 1a-hydroxytuberosone (**7**) were identified as pterocarpone and pterocarpanone subtypes of pterocarponoid compounds, respectively, from this plant [48–51].

However, in the more complex picture of isoflavonoid biogenesis and detoxification process, the occurrence of quantitatively minor isoflavone and pterocarponoid compounds with varying substitution patterns, incorporating hydroxyl, methoxyl, prenyl (C_3H_9), and analogs, oxidation(s) of substituent groups, and other chemical entities as substitutions, can be considered an outcome of aberrant biogenesis which seems to be running on a non-predetermined biogenetic plan and thereby producing new structural variants of the known classes and/or individual compounds as a specific result of response-mediated biogenetic action on demand because of the plant and its surroundings' interplay of either symbiotic nature or of hostile intentions. The presence of isoflavonoid glycosides with variations in glycone(s) positions, differences in glycone's esterified or etherified residues' nature in terms of alkyl structural types/chain length incorporation of other simple aromatics (*e.g*., substituted phenyls, cinnamic acids, etc.) and nonaromatic entities (*e.g*., terpenic, aliphatic residues) into the glycone part or attachment/fusion in aglycone moiety itself may also be an indication of response-mediated natural deviation from the planned biogenetic program. An array of plant products with biogenetically plausible and/or structurally varying isoflavonoid and pterocarponoids and their glycoside(s) for use as plant defencins, antifeedants, plant-antibiotics (phytoalexins), anti-inducers, and toxins are part of survival mechanism against variety of microbial pathogens, physicochemical and plant physiological stress factors in

Scheme 2. Enzyme-mimetic chemical conversions.



Reagents: (a) $NaBH_4$-MeOH, RT, (b) AcOH, (c) $Pb(OAc)_4$, AcOH, 0°C, (d) $K_4Fe(CN)_6$, (e) $NaIO_4$, (f) Zn-AcOH, RT, (g) CH_3I, K_2CO_3, (h) BBr_3, -78 °C, (i) DDQ, C_6H_6, Reflux (j) $(CH_3)_2O$, H_2O, $K_2S_2O_8$, (k) $Pb(OAc)_4$, AcOH, 0°C.

and around the plant ecosystem, wherein the demarcation between routine biogenesis and stimuli-responsive generation of natural products is hard to distinguished. Moreover, the biogenesis of an entirely different chemical class of compound produced from the biogenetic precursor-pool of common chemical entities but beyond the established biogenetic plan of producing the chemical markers and other species-specific compounds including compounds involved or produced as a function of plants' normal physiological conditions dictated by its genetic make-up, is probably because of earliest response towards the external or consequent internal stimuli passivation based upon its severity for the plant survival.

Thus, the isolation of puerarostan [52], puetuberosanol [53,54], dihydrodaidzein, *p*-hydroxybenzoic acid*, 3-methoxy-9-hydroxypterocarpene*, 3-O-methyldeoxytuberosin*, maltol*, 4′,7, 8-trihydroxyisoflavone* [55], puerarin, daidzin, 4′-hydroxy–7-O-rhamnosyl-isoflavone*, and an unidentified dimeric isoflavonoid product ($M^{+\cdot}$678) found during this study (*new to this plant) as well as the reported presence of 4′,6"di-O-acetylpuerarin [56] from *P. tuberosa* are noteworthy for their possible roles in plant microbe interplay and their further biogenetic interests with regard to the enzymatic participation in biochemical duress conditions of external stimuli. Herein, an attempt is made to find the common chemical ground for the major and characteristic chemical marker pterocarponoid constituents of *P. tuberosa* and mimic the different product-based enzymatic out-comes of the biogenesis to understand the analogy between the chemical and enzymatic transformations and their feasibilities at chemical levels.

The correlation of major isoflavones and pterocarponoid chemical marker compounds representing the advancing biogenetic transformation sequence among the 4′,7-dioxygenated isoflavone and 3,9-dioxygenated-2,2-dimethylchromeno-pterocarponoid classes are

chemically mimicked (Scheme 2) by formation of deoxytuberosin (**4**) from puerarone (**3**) [52,57] through C-3,4 α,β-unsaturated carbonyl's reduction and subsequent cyclization. The conversion of pterocarpan to pterocarpan-6a-ol is mimicked on template molecule, 3,9-dimethoxypterocarpan (**11**) after repeated attempts to produce tuberosin (**6**) or isotuberosin (**5**) [58] from deoxytuberosin (**4**) failed. The oxidative conversion of 3,9-dimethoxypterocarpan (**11**) to 3, 9-dimethoxypterocarpan-6a-ol (**12**) is carried out using lead tetra acetate-aqueous acetic acid. The conversion of tuberosin (**6**) to yield 1a-hydroxytuberosone (**7**) is achieved from sodium periodate after many attempts with Fremy'salt, thallium(III) triperchlorate, and thallium (III) triflouro acetate were unsuccessful to produce the desired compound, (**7**). The further transformations to 3-O-methyldeoxytuberosin (**4a**) from deoxytuberosin (**4**), 3-O-methylanhydrotuberosin (**8a**) from anhydrotuberosin (**8**) and vice versa (**8a** to **8**) is mimicked using methyl iodide for the methylation step in an analogy to the biogenesis of 3-O-methylpterocarpan and 3-O-methylpterocarpene from pterocarpan and pterocarpene, whereas boron tribromide [59] (Hazard!) was used for the demethylation step to produce back the pterocarpene (**8**) from (**8a**). The pterocarpone, tuberostan (**9**) is obtained by DDQ oxidation of 3-O-methylanhydrotuberosin (**8a**)—a known method [43]. However, attempts to produce pterocarpene, anhydrotuberosin (**8**) from pterocarpan, deoxytuberosin (**4**) by dehydrogenation and production of 4′,7,8 trihydroxyisoflavone (**10**) from 4′,7-dihydroxyisoflavone (daidzein) (**1**) by potassium persulfate oxidation in acetone-water did not yield the desired products.

This study provided the chemical correlation among the major isoflavone and kudzu vine's characteristic chemical marker pterocarponoid class of compounds with a view on biogenetic interrelationship and helped in establishing the chemical analogy for the enzyme-mimetic conversions of these compounds and their natural encounter in the plant as part of bioecology of plant microbe interactions and detoxification processes.

EXPERIMENTAL

General. The melting points are uncorrected and expressed in degree Celsius. The UV spectra were recorded on Hitachi-320 spectrophotometer and IR spectra on Perkin-Elmer 157 instrument as KBr pellets, unless otherwise stated. The PMR and mass spectra were recorded on Brucker WM 400-MHz spectrometer and Jeol JMS D-300 instrument in EI mode at 70 eV, respectively. The precoated silica gel TLC plates (Merck, Darmstadt) were used and visualized by short UV wavelength and/or on spraying with 2N sulphuric acid solution of ceric sulfate and subsequent heating.

Enzyme-mimetic chemical conversion of isoflavone and pterocarponoid constituents. The fresh tubers collected from Shahdol forests, MP, India were extracted with 95% aqueous ethanol under 40°C overnight, evaporated to dryness under *vacuum* as a red-brown gelly and partitioned between aqueous and hexane, chloroform, *n*-BuOH fractions and residual water portion. A part of the chloroform soluble fraction was repeatedly column chromatographed over silica-gel (23–400 mesh) in increasing polarities of hexane and EtOAc, neat EtOAc, and EtOAc-MeOH. All the isolated new and known products were well-characterized by MS, PMR, elemental analysis, specific rotations, IR and UV spectro-analytical methods, and circular dichroism measurements wherever needed and with thorough comparisons of m.p., mixed m.p., co-IR, co-UV determinations with the known reference standards of single spot TLC purity samples (elementally pure) obtained commercially or isolated and characterized during the course of this study (Table 1).

Puerarone (3) to Deoxytuberosin (4). To a solution of puerarone (**3**, 25 mg) in dry methanol (10 mL), sodium-borohydride (50 mg) was added and stirring continued, after 30 min, solvent was removed to furnish a residue, which was diluted with water, acidified with 5% aq. HCl, and extracted with chloroform (2 × 25 mL). The combined organic layers were dried over anhydrous sodium sulphate and conc. to give isoflavan-4-ol as oil. UV (MeOH): 230, 280, 290, 320 nm. MS (*m/z*): 340 (M+), 187 and 138. To the isoflavan-4-ol dissolved in dichloromethane (20 mL) was added glacial acetic acid (1.0 mL) and RM refluxed at water bath for 1 h. Solvent evaporated under vacuum to yield a solid, which was PLC purified to give deoxytuberosin (**4**), identical in all respects to the natural sample, amorphous solid, m p., 205°C, Co-TLC, Co-UV, and Co-IR., UV(MeOH): 220, 280, 330, and 310 nm. UV (MeOH + NaOH): 220, 282, 315, and 347 nm. IR (KBr): 3400 (br), 1590, 1260, 1045, 890 cm^{-1}. Elem. Anal., calculated for $C_{20}H_{18}O_4$: C 74.52, H 5.63, O 19.85, observed: C 74.54, H 5.66, O 19.89

Deoxytuberosin (4) to 3-O-methyl-deoxytuberosin (4a). Deoxytuberosin (**4,** 20 mg) on methylation in refluxing acetone (20 mL) with methyl iodide (0.5 mL) and anhydrous K_2CO_3 (30 mg) gave an amorphous solid on work up, which was identified as 3-O-methyl-deoxytuberosin (**4a,** 12 mg) on comparisons with the natural sample., m p., 187°C, Co-TLC, Co-UV, and Co-IR, UV (MeOH): 305, 335, and 322 nm. UV (MeOH + NaOH): 245, 282, 345, and 367 nm. IR (KBr): 1596, 1240, 1065, 790 cm^{-1}. Elem. Anal., calculated for $C_{21}H_{20}O_4$: C 74.98, H 5.99, O 19.03, observed: C 75.02, H 6.01, O 19.06.

Tuberosin (6) to 1a-hydroxytuberosone (7). To tuberosin (**6**, 30 mg), excess sodium periodate (20 mg) in water (50 mL) was added and stirred for 10 h. The light-brown mixture evaporated to 1/3 volume, extracted with diethyl ether (2 × 25 mL), dried over anhydrous sodium sulfate and concentrated to an oily mass, which was purified by PLC to give 1a-hydroxytuberosone, identical to natural sample (**7**, 22 mg), m. p. 133°C, (α)D +117° (c = 0.2, MeOH). Co-TLC, Co-UV, Co-IR, mixed m.p., UV (MeOH): 222, 280, 308, and 325 nm. IR (Neat): 3450, 1665, 1485, 1020, and 760 cm^{-1}. Elem. Anal., calculated for: $C_{20}H_{18}O_6$: C 67.79, H 5.12, O 27.09, observed. C 67.82, H 5.14, O 27.11.

1a-Hydroxytuberosone (7) to tuberosin (6). A mixture of 1a-hydroxytuberosone (**7**, 10 mg), Zn-dust (20 mg) and glacial acetic acid (4.0 mL) was vigorously stirred at RT for 2 h and

Table 1
Spectro-analytical data of major compounds.

Sr. No.	Compound	Molecular formula, MW (M^+) and mass peaks (*m/z*)	NMR data: ^{1}H NMR:Solvent-d_x,TMS as internal reference, chemical shifts interchangeable and expressed in δ values, coupling constants in Hz
1	Daidzein	$C_{15}H_{10}O_4$, 254 (M+), 137, 118	Acetone-d_6 + DMSO-d_6: 6.65 - 6.96 (m, 3H, C3′H, C5′H, C6H), 7.04 -7.30 (m, 2H, C2′H, C8H), 7.88 (d, 1H, J = 8.5 Hz, C5H), 8.76 (s, 1H, C2H).
2	Neobavaisoflavone	$C_{20}H_{18}O_5$, 338 (M+), 323, 253, 161	Acetone-d_6: 6.56 (m, 3H, C2′H, C6′H, C6H), 6.99-7.15 (m, 2H, C5′H, C8H), 7.79 (d, 1H, J = 8.5 Hz, C5H), 8.89 (s, 1H, C2H).
3	Puerarone	$C_{20}H_{16}O_5$, 336 (M+), 321 (M^+-CH_3), 170, 134	Acetone-d_6: 1.40 (br s, 6H, 2 × CH_3), 5.62 (d, 1H, J = 10.0 Hz, C13H), 6.35 (d, 1H, J = 10.0 Hz, C12H), 6.37 (s, 1H, C7H), 7.02 (d, 1H, J = 2.0 Hz, C8H), 7.04 (s,1H, C10H), 7.20 (dd, 1H, J = 8.5, 2.0 Hz, C6H), 8.143 (d, 1H, J = 8.5 Hz, C5H), 8.34 (s, 1H, C2H).
4	Deoxytuberosin	$C_{20}H_{18}O_4$, 322 (M^+), 307, 173, 149, 136	Acetone-d_6: 1.24 (br s, 6H, 2 × CH_3, isoprenyl), 3.22 (m, 1H, C6H), 4.02 (m, 2H, C6aH × 2), 5.16 (m, 1H, C11aH), 5.64 (d, 1H, J = 10.0 Hz, C13H), 6.37 (d, 1H, J = 10.0 Hz, C12H), 6.67 (dd, 1H, J = 8.5, 2.5 Hz, C2H), 7.00 (s, 1H, C10H), 7.22 (d, 1H, J = 2.5 Hz, C4H), 7.38 (s, 1H, C7H), 7.42 (d, 1H, J = 8.5 Hz, C1H).
5	1a-Hydroxytuberosone	$C_{20}H_{18}O_6$, 354 (M+), 339, 320, 305, 201, 163	Acetone-d_6: 1.37 (s, 6H, 2 × CH_3), 4.40 (d, 1H, C6α-H, J = 11 Hz), 4.80 (s, 1H, C11 αH), 5.12 (d, 1H, C6βH, J = 11 Hz), 5.30 (d, 1H, C4H, J = 2.0 Hz), 5.60 (d, 1H, C13, J = 10 Hz), 6.02 (dd, 1H, C2H, J = 10.0, 2.0 Hz), 6.10 (s, 1H, C10H), 6.42 (d, 1H, C12H, J = 10 Hz), 6.90 (d, 1H, C1H, J = 9 Hz), 7.10 (s, 1H, C7H).
6	Tuberosin	$C_{20}H_{18}O_5$, 338 (M+), 320,305,149.	$CDCl_3$: 1.37 (s, 6H, 2 × CH_3), 4.00 (d, 1H, J = 11.0 Hz, C-6H), 4.02 (d, 1H, J = 11.0 Hz, C6′H), 5.20 (s, 1H, C11H), 5.60 (d, 1H, J = 10.0 Hz, C12H), 6.25 (s, 1H, C7H), 6.26 (d,1H, J = 2.0 Hz, C4H), 6.40 (d, 1H, J = 10.0 Hz, C13 H), 6.50 (dd, 1H, J = 8.5, 2.0 Hz, C2H), 7.02 (s, 1H, C10 H), 7.25 (d, 1H, J = 8.5 Hz, C1H).
7	3-O-Methylanhydro-tuberosin	$C_{21}H_{18}O_4$, 334 (M+), 319 (M+-CH_3, 100 %), 295	$CDCl_3$: 1.46 (s, 6H, 2 × CH_3), 3.81 (s, 3H, OCH_3), 5.52 (s, 2H, C-6H), 5.63 (d, 1H, C-13H, J = 10 Hz), 6.40 (d, 1H, C12H, J = 10.0 Hz), 6.50 (dd, 1H, C2H, J = 8.5, 2.5 Hz), 6.52 (d, 1H, C4H, J = 2 Hz), 6.70 (s, 1H, C10H), 7.00 (d, 1H, C1H, J = 8.5 Hz), 7.14 (s, 1H, C7H).
8	Anhydrotuberosin	$C_{20}H_{16}O_4$ 320 (M+), 305, 295, 136	$CDCl_3$:1.43 (s, 6H, 2 × CH_3), 5.53 (s, 2H, C6H), 5.70 (d, 1H, C13H, J = 10 Hz), 6.45 (d, 1H, C12H, J = 10 Hz), 6.50 (d, 1H, C4H, J = 2 Hz), 6.54 (dd,1H, C2H, J = 8.5, 2.5 Hz), 6.82 (s, 1H, C10H), 7.10 (s, 1H, C 7H), 7.30 (d, 1H, C1H, J = 8.5 Hz).
9	Tuberostan	$C_{21}H_{16}O_5$, 348 (M+), 347, 333(100%), 318, 262, 202, 167, 145.	$CDCl_3$:1.43 (brs, 6H, 2 × CH_3), 3.81 (s, 3H, OCH_3), 5.75 (d, 1H, 10 Hz, C13H), 6.50 (d, 1H, 10 Hz, C12H), 7.15 (m, 2H, C3H, C5H), 7.30 (s, 1H, C10 H), 7.95 (dd, 1H, J = 8.5, 2.5 Hz, C2H), 8.02 (s, 1H, C7H).
10	8-Hydroxy daidzein*	$C_{15}H_{10}O_5$, 270 (M^+), 152, 118	Acetone-d_6: 6.70-6.90 (m, 4H, ring-B, C3′H, C4′H, C5′H, C6′H), 7.35 (d, 1H, C6H, J = 8.5 Hz), 7.99 (d, 1H, C8H, J = 8.5 Hz), 8.00 (s,1H, C2H).
11	3,9-Dimethoxy-pterocarpan	$C_{17}H_{16}O_4$, 300 (M+), 285, 284, 256, 241, 224, 186, 163, 148	$CDCl_3$: 3.58 (s, 3H, OCH_3), 3.22 (m, 1H, C 6a), 3.99 (m, 2H, C 6H), 5.11 (m, 1H, C 11aH), 6.64 -6.77 (m, 2H, C2H, C8H), 6.86 (s, 1H, C10H), 7.00 (d, 1H, J = 2.0 Hz, C4H), 7.22 (s, 1H, C7H), 7. 26 (d, 1H, J = 8.5 Hz, C1H).
12	6a-Hydroxy-3, 9-dimethoxy-pterocarpan	$C_{17}H_{16}O_5$, 300 (M+), 285, 284, 256, 241, 224,186,163, 148.	$CDCl_3$: 3.77 (s, 6H, 2 × OCH_3), 4.55 (brd, 2H, C6H, J = 11.5, Hz), 5.72 (s, 1H, C11aH), 6.65 (m,2H, C8H, C10 H), 7.01 (m, 2H, C3H, C5H), 7.55 (dd, 1H, C2H, J = 8.5, 2.0 Hz), 7.78 (d, 1H, C7H, J = 8.6 Hz).
13	Isotuberosin*	$C_{20}H_{18}O_6$, 338(M+), 323, 305, 295, 279, 256, 149.	$CDCl_3$: 1.37 (s, 6H, 2 × CH_3), 4.02 (d, 1H, J = 11.0 Hz, C-6H), 4.05 (d, 1H, J = 11.0 Hz, C6′H), 5.25 (s, 1H, C11H), 5.54 (d, 1H, J = 10.0 Hz, C12H), 6.20 (s, 1H, C7H), 6.30 (d, 1H, J = 2.0 Hz, C4H), 6.38 (d, 1H, J = 10.0 Hz, C13 H), 6.55 (dd, 1H, J = 8.5, 2.0 Hz, C2H), 7.05 (s, 1H, C10 H), 7.28 (d, 1H, J = 8.5 Hz, C1H)., $[\alpha]_D$ +11.0 (c =1, acetone), Circular Dichroism, MeOH: + 262, 284, 290, 340, Oil.
14	3-O-Methyl-deoxytuberosin*	$C_{21}H_{20}O_4$, 336(M^+), 321 (M+ -CH3, 100 %), 307, 149.	$CDCl_3$: 1.37 (br s, 6H, 2 × CH_3, isoprenyl), 3.25 (m, 1H, C6H), 3.55 (s, 3H, Aryl-OCH_3), 4.00 (m, 2H, C6a H × 2), 5.20 (m, 1H, C11aH), 5.70 (d, 1H, J = 10.0 Hz, C13H), 6.42 (d, 1H, J = 10.0 Hz, C12H), 6.70 (dd, 1H, J = 8.5, 2.5 Hz, C2H), 7.00 (s, 1H, C10H), 7.20 (d, 1H, J = 2.5 Hz, C4H), 7.41 (s, 1H, C7H), 7.42 (d, 1H, J = 8.5 Hz, C1H).

* New to plant.

water (15 mL) was added slowly followed by extraction with chloroform (2 × 15 mL). The combined extracts were dried over anhydrous sodium sulfate and solvent removed under *vacuum* to afford tuberosin (6 mg). m.p. 211°C, UV (MeOH): 220, 280, 312, 318, and 325 nm., IR: 3250, 1610, 1560, 1380, 1160, 1040, 800, 760 cm^{-1}. Elem. Anal., calculated for $C_{20}H_{18}O_5$: C 70.99, H 5.36, O 23.64, observed. C 71.00, H 5.36, O 23.65.

Anhydrotuberosin (8) to 3-O-methylanhydrotuberosin (8a). To a solution of anhydrotuberosin (**8**, 20.0 mg) in dry acetone (25 mL) was added methyl iodide (1.0 mL), potassium carbonate (25 mg), and reaction mixture refluxed for 1 h, cooled, solvent evaporated to furnish a residue, which was dissolved in diethyl (20 mL), washed with water, organic layers dried over anhydrous sodium sulfate, and evaporated to give the product (**8a**, 12 mg), identical in all respect to natural sample. m.p. 165°C, TLC, Co-T LC UV, FT-IR, Co-UV, Co-IR, mixed m.p., UV (MeOH, max): 230, 245, 332, 360 nm. Elem. Anal., calculated for: $C_{21}H_{18}O_4$: C 75.43, H 5.43, O 19.14., Observed. C 75.45, H 5.44, O 19.16.

3-O-Methylanhydrotuberosin (8a) to anhydrotuberosin (8). To a stirred solution of cooled (dry ice-acetone, −78°C) 3-O-methylanhydrotuberosin (**8a**, 20 mg) in dry dichloromethane (15 mL) was added boron tri bromide in excess (2 mL) *via* syringe and stirring continued for 30 min. The reaction mixture was allowed to warm to RT and the concentrated residue was washed with water, $CHCl_3$ extracted, dried over anhydrous sodium sulfate and concentrated under *vacuum* to give the product (**8**, 12 mg), identical to natural sample in all respects. m.p. 187°C, Co-TLC, Co-UV, Co-IR, mixed m.p., UV (MeOH): 238, 262, 330, 350 nm. Elem. Anal., calculated for $C_{20}H_{16}O_4$: C 74.99, H 5.03, O 19.98, observed. C 75.01, H 5.05, O 20.01.

3-O-Methylanhydrotuberosin (8a) to tuberostan (9). To a stirring solution of 3-O-methyl anhydrotuberosin (**8a**, 25 mg) in benzene (10 mL) was added 1,4-dichlorodicyanoquinone (DDQ) (50 mg) and refluxed for 24 h, RM cooled to RT, filtered, water added to filtrate, extracted with chloroform (2 × 20 mL), and dried over anhydrous sodium sulfate and solvent evaporated to give product (**9**, 23 mg) as white solid, identical in all respect to natural sample. Co-TLC, Co-UV, Co-IR., mixed m.p., 220°C, UV (MeOH): 220, 245, 252, 352, and 366. IR (KBr): 1730, 1640, 1422, 1120, 960, and 745 cm^{-1}. Elem. Anal., calculated for $C_{21}H_{16}O_5$: C 72.41, H 4.63, O 22.96, Observed. C 73.43, H 4.66, O 22.99.

3,9-Dimethoxypterocarpan (11) to 6a-Hydroxy-3,9-dimethoxypterocarpan (12). To a stirring solution of 3,9-dimethoxypterocarpan (**11**, 25 mg) in wet dichloromethane (10 mL) at 0°C was added lead (iv) tetra acetate (10 mg), acetic acid (1.0 mL) and reaction mixture stirred for 1 h at 0°C. Solvent removal afforded a brownish residue, which was dissolved in ethyl acetate (10 mL), water washed and organic layer dried over anhydrous sodium sulfate. Solvent removal under *vacuum* afforded a residue, which on PLC purification in chloroform:-hexane (1:1, v/v) gave the product as viscous oil (12 mg). UV (MeOH): 215, 230, 280, and 330 nm. IR (Neat): 3300 (br), 1620, 1590, 1475, 1320, 1265, 840, and 760 cm^{-1}. Elem. Anal., calculated for $C_{17}H_{16}O$: C 67.99, H 5.37, O 26.64, observed. C 67.98, H 5.39, O 26.65.

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