Structure Elucidation of Kwakhurin, a New Prenylated Isoflavone from *Pueraria mirifica* Roots

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Leguminosae, *Pueraria,* Isoflavonoid, Isoflavone, Phenolic Compound

An isoflavonoid obtained from dried roots of the Thai legume *Pueraria mirifica* has been identified by chemical and spectroscopic methods as 7,2′,4′-trihydroxy-5′-methoxy-6′-(3,3-dimethylallyl)isoflavone (kwakhurin).

Introduction

In two earlier papers [1, 2] we reported that the tuberous roots of *Pueraria mirifica* Airy Shaw and Suvatabandhu (Leguminosae-Papilionoideae; tribe Phaseoleae) contained the isoflavonoids daidzein (7,4′-dihydroxyisoflavone, **1**), daidzin (daidzein-7-O-glucoside), puerarin (daidzein-8-C-glucoside), genistein (5,7,4′-trihydroxyisoflavone) and coumestrol (3,9-dihydroxycoumestan) as well as a novel 6″-O-apioside (mirificin) of puerarin. Apart from these compounds, methanolic root extracts also yielded three other coumestans and a prenylated 5-deoxyisoflavone denoted PM-7 in ref. [1]. Although the latter isoflavonoid was found to have A- and C-rings identical with those of daidzein (**1**), and a B-ring possessing two OH groups, an OMe substituent and a 3,3-dimethylallyl (prenyl) sidechain, the amount available for investigation (5 mg) was insufficient to allow complete structure elucidation. Recently, however, we recovered a comparatively large quantity (approx. 230 mg) of PM-7 from an isoflavonoid-rich fraction originally obtained during the isolation of miroestrol, a potent non-flavonoid oestrogen [3, 4], from *P. mirifica* roots. Re-examination of this material has now resulted in the identification of PM-7 as 7,2′,4′-trihydroxy-5′-methoxy-6′-(3,3-dimethylallyl) isoflavone (**2**), details of which are described below. We propose that **2** should be called kwakhurin from the Thai native name *(kwao khua)* for *P. mirifica*.

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Results and Discussion

As reported in our previous paper [1], kwakhurin ($[M]^+$ 368) was initially identified as a 5-deoxyisoflavone from its daidzein-like appearance on Si gel thin-layer plates viewed under long wavelength (365 nm) UV light (pale blue, rapidly intensifying upon fuming with NH_3 vapour [5]). Three aromatic OH groups were revealed by the formation of both a trimethyl ether ($[M]^+$ 410, **3**) and a triacetoxy derivative ($[M]^+$ 494, **4**). In the ^1H NMR spectrum (acetone-d_6), a low-field singlet at δ 7.84 was assigned to H-2, whilst mono (OH) substitution of ring A was evident from the NaOAc-induced shift of the UV (MeOH) maximum at 249 nm (C-7 OH [5]), the prominent MS fragment at *m/z* 137 (*m/z* 151 in the case of trimethyl ether **3**), and the characteristic NMR signals at δ 8.02 (d, $J = 8.5$ Hz, H-5), 6.98 (dd, $J = 8.5$ and 2.4 Hz, H-6) and 6.91 (d, $J = 2.4$ Hz, H-8) which resembled those afforded in DMSO-d_6 by the A-ring protons of **1** [6]. Thus kwakhurin possesses part structure **a** (Fig. 1) with ring B carrying two OH groups as well as a 3,3-dimethylallyl sidechain (δ 1.39 and 1.49, both s, 2 × Me; δ 5.01 br t, $J = ca$ 5.7 Hz, C<u>H</u>; δ 3.09

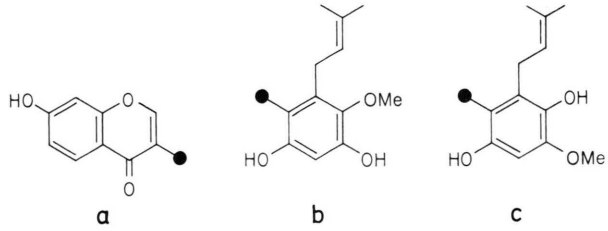
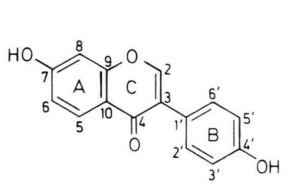


Fig. 1. Part structures discussed in the text.



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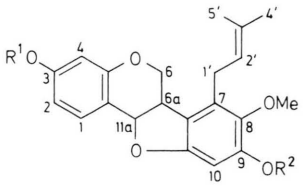

5: $R^1 = R^2 = H$
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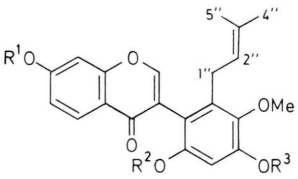
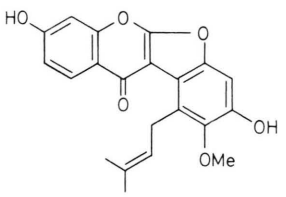


2: $R^1 = R^2 = R^3 = H$
3: $R^1 = R^2 = R^3 = Me$
4: $R^1 = R^2 = R^3 = Ac$
6: $R^1 = R^2 = R^3 = Et$



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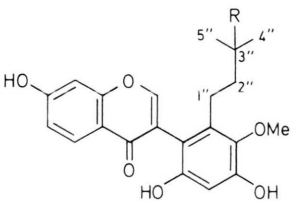
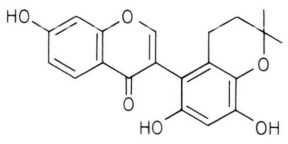


9: R = OH
10: R = OCOH
11: R = OAc
13: R = Cl



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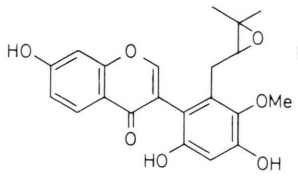

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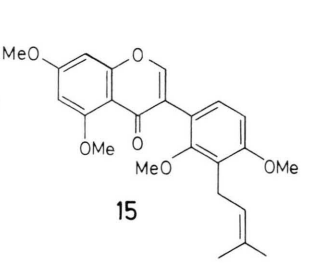

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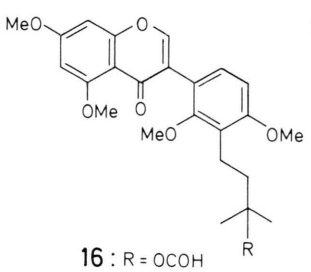
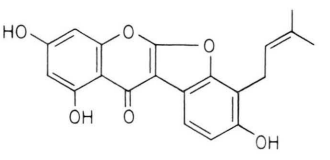


16: R = OCOH
17: R = OH



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dd, $J = 14.7$ and 7.3 Hz, H_a of $C\underline{H}_2$; δ 3.31 dd, $J = 14.7$ and 6.1 Hz, H_b of $C\underline{H}_2$), and a single OMe substituent (δ 3.71 s).

One of the B-ring OH groups was readily assigned to C-2′ as treatment of kwakhurin with $NaBH_4$ afforded the dihydroxy pterocarpan **5** ($[M]^+$ 354) in good yield. Significantly, the lone D-ring proton of **5** exhibited a ^1H NMR chemical shift value (δ 6.26; acetone-d_6) almost identical with that given in the same solvent by H-10 (δ 6.29 [7]) of 3,9-dihydroxypterocarpan (*cf.* δ values of 7.13 and 6.37 obtained respectively for H-7 and H-8 of the latter pterocarpan [7]), a result which suggested that in **2** the equivalent 3′-position was unsubstituted.

Location of the 3,3-dimethylallyl attachment at C-6′ was first deduced from the ^1H NMR spectrum of **2** which, as mentioned above, revealed two 3H singlets (δ 1.39 and 1.49) attributable to the terminal Me groups. In the spectra of many A- or B-ring prenylated isoflavones (*e.g.* luteone [prenyl at C-6], licoisoflavone A = 'phaseoluteone' [prenyl at C-3′] and 5,7,4′-trihydroxy-3′,5′-di-(3,3-dimethylallyl) isoflavone), these signals typically appear between δ 1.60 and 1.85 [8–10]. However, as in **2**, the sidechain methyls of piscidone, a 6′-prenylated isoflavone from *Piscidia piscipula* (= *P. erythrina*) [11], also resonate at unusually high field (δ 1.43 and 1.53 in acetone-d_6 [12]), and this indicates that kwakhurin is similarly substituted at C-6′.

Further support for a 6′-prenyl group was obtained by conversion of kwakhurin to the coumaronochromone derivative **8** ($[M]^+$ 366). Apart from the absence of an H-2 singlet, the ^1H NMR spectrum of **8** was notable in that the 1″-$C\underline{H}_2$ signal (δ 4.26 br d, $J = 6.6$ Hz) appeared at much lower than normal field (*cf.* data for **2** [sidechain $C\underline{H}_2$ at approx. δ 3.20] and **5** [δ 3.39] in acetone-d_6, and for the model coumaronochromones trimethyl-lisetin [δ 3.59] and lupinalbin D [**18**; δ 3.60] in $CDCl_3$ and acetone-d_6 respectively [11, 13]). This observation can be explained if the sidechain methylene of **8** is strongly deshielded by the carbonyl substituent, an effect which is inconsistent with prenylation at any position other than C-6′.

All known *Pueraria* isoflavones are oxygenated at C-4′ [1, 14]. Assuming that kwakhurin is similarly oxygenated, the B-ring can be represented by either part structure **b** or **c** since failure to afford a blue or purple-blue Gibbs test colour [15, 16] is indicative of substitution *para* to the 2′-hydroxyl group. A decision in favour of **b** (OH at C-4′ as in other isoflavones from *P. mirifica* [1, 2]) rather than **c** was eventually made following a series of spectroscopic and chemical studies, details of which are given below.

a) Spectroscopic investigations

It has been reported [17] that the presence of an OMe substituent *ortho* to CH on an aromatic ring can be detected by a ^{1}H NMR shift of the OMe signal when the solvent is changed from $CDCl_3$ to C_6D_6. An up-field shift of 0.30 ppm or more indicates a free *ortho* CH whilst a shift of 0–0.2 ppm is evidence for *ortho* substitution. This procedure, which is most satisfactorily applied to fully alkylated compounds, has been used in the characterization of philenopteran (pterocarpan) from *Lonchocarpus laxiflorus* [17], and sepiol (isoflavene) from *Gliricidia sepium* [18]. As shown in Table I, the OMe signal of tri-O-ethyl-kwakhurin (**6**) exhibited a negligible (0.01 ppm) shift upon changing the solvent from $CDCl_3$ to C_6D_6, and an exactly comparable result was obtained when the di-O-ethylpterocarpan (**7**) was used instead of **6**. The lack of an OMe shift associated with **6** and **7** is in marked contrast to the effect on the CH_2 signals of the ethoxy substituents in these compounds where shifts considerably greater than 0.3 ppm were observed (Table I). Such effects, and similar ones involving the CH_3 portion of the ethoxy units (Table I), are consistent with an isoflavone structure (**2**; **a** + **b**) capable of yielding an ethyl ether in which all three ethoxy groups have *ortho*-unsubstitution.

Table I. ^{1}H NMR solvent shifts of the methoxy and ethoxy signals of kwakhurin triethyl ether (**6**) and kwakhurin pterocarpan diethyl ether (**7**).*

Compound/substituent	Solvent shift (δ value) $CDCl_3$	C_6D_6	Δδ
Kwakhurin triethyl ether (**6**)			
OMe	3.79s	3.80s	−0.01
$3\times OCH_2CH_3$	3.93q	3.35q	0.58
	4.09q	3.74q	0.35
	4.41q	3.74q	0.40
$3\times OCH_2CH_3$	1.19t	0.97t	0.22
	1.47t	1.06t	0.41
	1.55t	1.21t	0.34
Kwakhurin pterocarpan diethyl ether (**7**)			
OMe	3.76s	3.75s	0.01
$2\times OCH_2CH_3$	4.00q	3.55q	0.45
	4.02q	3.56q	0.46
$2\times OCH_2CH_3$	1.40t	1.05t	0.35
	1.43t	1.07t	0.36

* Full ^{1}H NMR data for compounds **6** and **7** are given in the Experimental section.

Two other lines of spectroscopic evidence also supported part structure **b**. First, in the ^{13}C NMR spectrum of **2** the chemical shift of the OMe (δ 61.1) was consistent with the presence of substituents in both *ortho*-positions [19]. Secondly, in a ^{1}H NMR NOE difference experiment, irradiation of the OMe resonance clearly enhanced the 2″-CH signal but had no effect on the aromatic proton signal at δ 6.39 (B-ring, H-3′). Similarly, irradiation of the OMe (δ 3.75) of pterocarpan **5** only resulted in enhancement of the sidechain CH signal (δ 5.19). In contrast to these results, a positive NOE on H-3′ (rather than on the sidechain CH) would have been expected if the B-ring of kwakhurin possessed structure **c**.

b) Chemical investigations

Isoflavones with an OH group (or groups) located *ortho* to a 3,3-dimethylallyl sidechain are known to yield the corresponding cyclo product(s) when heated with acid [8, 20, 21]. However, all attempts to cyclize kwakhurin were unsuccessful, with the compound instead being found to afford one or more sidechain addition products.

Thus, when heated with formic acid [22] **2** gave kwakhurin hydrate (**9**) and a formic acid addition product (**10**) whilst treatment with glacial acetic acid and conc. H_2SO_4 [11, 20] afforded **9** on one occasion and **9** plus a diacetate, an acetic acid addition product (**11**) and demethyl-cyclokwakhurin (**12**) on another. Isoflavone **12** and an HCl addition product (**13**) were obtained after heating **2** with $AlCl_3$ in acetonitrile [23]. These observations, and subsequent results which revealed that kwakhurin was unaffected by *p*-toluenesulphonic acid [24], whilst merely being converted to epoxide **14** (rather than to a cyclic dihydrofurano-isoflavone) by *m*-chloroperoxybenzoic acid [25], are all in accord with

part structure **b** where sidechain cyclization is prevented by the OMe substituent at C-5′. Only when the OMe group was first removed by heating with $AlCl_3$ in acetonitrile did cyclization occur. As expected, formic acid treatment of the model isoflavone licoisoflavone A tetramethyl ether (**15**), a compound with blocking OMe groups on either side of the prenyl attachment, similarly afforded a hydrate (**17**) and a formic acid addition product (**16**). The formation of a hydrate derivative (and addition products with MeOH and trifluoroacetic acid) has also been described for a 6′-prenylated isoflavone (6′-isopentenylpiscerythrone) from *Piscidia piscipula* [10] where, as in **2**, cyclization is prevented by an *ortho* (C-5′) located OMe group.

Together with the chemical and spectroscopic results described earlier, the above data firmly establish the structure of kwakhurin (compound PM-7 [1]) as 7,2′,4′-trihydroxy-5′-methoxy-6′-(3,3-dimethylallyl)isoflavone. Although many prenylated isoflavones have now been identified [14] only two, in addition to kwakhurin, are known to possess a 6′-prenyl attachment, these being piscidone [10, 11, 14] and 6′-isopentenylpiscerythrone [10] from the root bark of *P. piscipula*. Despite its phenolic nature and possession of both prenyl and OMe substituents, kwakhurin (100 μg) lacked antifungal activity when tested (thin-layer plate bioassay [16, 25]) against the growth of *Cladosporium herbarum* Fr. In contrast, a slight decrease in fungal growth was caused by only 1 μg of luteone [5,7,2′,4′-tetrahydroxy-6-(3,3-dimethylallyl)isoflavone], with prominent inhibition zones being evident at applied levels of 25 and 50 μg [12, 20].

Experimental

Roots of *Pueraria mirifica* Airy Shaw and Suvatabandhu were collected in Thailand [1, 2]. Si gel TLC of kwakhurin and its derivatives was carried out on Merck pre-coated, glass-backed plates (F-254, layer thickness 0.25 or 0.5 mm) in one or more of the following solvent systems: *BE* = C_6H_6–EtOAc, *CM* = $CHCl_3$–MeOH, *CHM* = $CHCl_3$–*n*-hexane–MeOH, *CAEM* = $CHCl_3$–acetone–EtOAc–MeOH, *HE* = *n*-hexane–EtOAc, and PEA = *n*-pentane–Et_2O–glacial HOAc (details of solvent ratios are given in the text). ^{1}H NMR spectra were run (TMS reference) at either 500 MHz (kwakhurin) or 100 MHz (all other compounds). Except where indicated, the signals integrated for 1H.

Isolation and purification of kwakhurin

An EtOAc extract of powdered *P. mirifica* roots (approx. 10 kg) was obtained using the procedure described by Jones and Pope [3]. This extract (50 g) was then fractionated on a 10 cm i.d. Celite column using as eluant the upper (mobile) phase of the immiscible solvent system toluene–EtOAc–MeOH–H_2O (6:4:5:5) [4]. Kwakhurin was found principally in fraction 5. After removal of solvent, the yellow-brown residue (5 g) was taken up in MeOH and adsorbed onto Si gel, the resulting powder then being applied to a dry-pack Si gel column (Merck, Type 7734; 400 g) prior to elution with $CHCl_3$–MeOH (20:1). Eluates were monitored for kwakhurin by Si gel TLC, and the appropriate fractions were bulked and reduced to dryness *in vacuo* (40 °C). Yield: approx. 23 mg of kwakhurin per kg dried *P. mirifica* root.

*7,2′,4′-Trihydroxy-5′-methoxy-6′-(3,3-dimethylallyl)isoflavone (**2**) (kwakhurin)*

UV maxima as lit. [1]. EI-MS (rel. int.): $[M]^+$ 368 (30), *m/z* 353 (4), 335 (4), 258 (4), 243 (6), 232 (14; intact B-ring fragment from RDA fission), 231 (7), 217 (5), 169 (10), 149 (8), 138 (8), 137 (100; A-ring fragment from RDA fission), 115 (5). ^{1}H NMR (acetone-d_6): δ 1.39, 1.49 (br s, both 3H, 4″- and 5″-Me), 3.09 (dd, J = 14.7 and 7.3 Hz, H_a of 1″-C$\underline{H}_2$), 3.31 (dd, J = 14.7 and 6.1 Hz, H_b of 1″-C$\underline{H}_2$), 3.71 (s, 3H, OMe), 5.01 (br t, J = 6.7 Hz, 2″-C$\underline{H}$), 6.39 (s, H-3′), 6.91 (d, J = 2.4 Hz, H-8), 6.98 (dd, J = 8.5 and 2.4 Hz, H-6), 7.84 (s, H-2), 8.02 (d, J = 8.5 Hz, H-5). NOE experiment: irradiation of the OMe signal enhanced only the 2″-C$\underline{H}$ resonance. ^{13}C NMR (acetone-d_6; 60 MHz): δ 17.6, 25.6 (C-4″ and -5″), 27.5 (C-1″), 61.1 (OMe), 102.4 (C-8), 103.1 (C-3′), 111.5 (unassignable C of B-ring), 115.4 (C-6), 118.7 (C-10), 121.4 (C-3), 124.8 (C-2″), 128.3 (C-5), 130.8 (C-3″), 136.3, 140.1, 151.4, 153.3 (all unassignable B-ring carbons), 155.5 (C-2), 159.0 (C-9), 163.0 (C-7), 176.6 (C-4).

*Kwakhurin trimethyl ether (**3**)*

Kwakhurin (6.6 mg) was refluxed with dimethyl sulphate (50 μl) and anhydrous K_2CO_3 (200 mg) in dry acetone (8 ml) for 2 h. After removal of K_2CO_3

(filtration) and solvent (*in vacuo*, 40 °C), the residue was chromatographed (Si gel TLC in CM, 50:1) to afford 5.4 mg of **3** (R_F 0.84). UV maxima as lit. [1]. EI-MS (rel. int.): $[M]^+$ 410 (97), *m/z* 409 (9), 395 (13), 379 (7), 367 (5), 363 (4), 354 (5), 341 (7), 339 (8), 311 (4), 271 (6), 260 (20; intact B-ring fragment from RDA fission), 259 (17), 245 (8), 229 (4), 228 (4), 191 (5), 190 (20), 177 (4), 152 (11), 151 (100; A-ring fragment from RDA fission). ^{1}H NMR (acetone-d_6): δ 1.40 (s, 3H, sidechain Me), 1.50 (d, J = 0.98 Hz, 3H, sidechain Me), 3.07 (dd, J = 14.2 and 7.1 Hz, H_a of 1″-C$\underline{H}_2$), 3.33 (dd, J = 14.2 and 6.4 Hz, H_b of 1″-C$\underline{H}_2$), 3.69, 3.73, 3.92, 3.98 (s, all 3H, 4 × OMe), 4.98 (br t, J = *ca* 7 Hz, 2″-C$\underline{H}$), 6.66 (s, H-3′), 7.05 (dd, J = 9.5 and 2.4 Hz, H-6), 7.05 (d, J = 2.4 Hz, H-8), 7.82 (s, H-2), 8.06 (d, J = 9.5 Hz, H-5).

*Kwakhurin triacetate (**4**)*

Kwakhurin (5.5 mg) was heated with a 1:1 mixture of acetic anhydride and pyridine at 80 °C for 3 h. The reaction mixture was then diluted with toluene and reduced to dryness *in vacuo* (40 °C). Si gel TLC (CM, 100:1) of the residue gave 7 mg of **4** (R_F 0.73). UV: λmax, nm: MeOH 235 sh, 295, 303. EI-MS (rel. int.): $[M]^+$ 494 (20), *m/z* 453 (16), 452 (54), 411 (10), 410 (40), 409 (8), 368 (10), 367 (9), 350 (8), 335 (7), 258 (8), 232 (21), 231 (14), 179 (48), 138 (8), 137 (100). ^{1}H NMR (acetone-d_6): δ 1.39 (br s, 3H, sidechain Me), 1.48 (d, J = 0.97 Hz, 3H, sidechain Me), 1.99 (s, 3H, OAc), 2.35 (s, 6H, 2 × OAc), 3.29 (br t − like, J = 7.6 Hz, 2H, 1″-C$\underline{H}_2$), 3.80 (s, 3H, OMe), 4.96 (br t, J = 6.8 Hz, 2″-C$\underline{H}$), 6.95 (s, H-3′), 7.30 (dd, J = 8.7 and 2 Hz, H-6), 7.45 (d, J = 1.7 Hz, H-8), 7.99 (s, H-2), 8.20 (d, J = 8.3 Hz, H-5).

*Kwakhurin triethyl ether (**6**)*

Kwakhurin (6.5 mg) was refluxed with diethyl sulphate (60 µl) and anhydrous K_2CO_3 (200 mg) in dry acetone (10 ml) for 2 h. Work up followed by Si gel TLC (CHM, 60:40:1) afforded 3.7 mg of **6** (R_F 0.22−0.30). UV: λmax, nm: MeOH 240 sh, 248, 293, 305 sh. EI-MS (rel. int.): $[M]^+$ 452 (100), *m/z* 424 (15), 423 (50), 391 (12), 288 (18; intact B-ring fragment from RDA fission), 287 (15), 285 (15), 259 (10), 169 (12), 166 (11), 165 (95; A-ring fragment from RDA fission), 137 (29). ^{1}H NMR ($CDCl_3$): δ 1.19, 1.47, 1.55 (t, each J = 7.1 Hz, all 3H, 3 × C$\underline{H}_3$ of EtO), 1.41, 1.54 (s, both 3H, 4″- and 5″-Me), 3.04 (dd, J = 14.3 and 7.8 Hz, H_a of 1″-C$\underline{H}_2$), 3.37 (dd, J = 14.3 and 6.4 Hz, H_b of 1″-C$\underline{H}_2$), 3.79 (s, 3H, OMe), 3.93, 4.09, 4.14 (q, each J = 7.1 Hz, all 2H, 3 × C$\underline{H}_2$ of EtO), 5.02 (br t, J = *ca* 7 Hz, 2″-C$\underline{H}$), 6.45 (s, H-3′), 6.84 (d, J = 2.4 Hz, H-8), 6.96 (dd, J = 8.8 and 2.4 Hz, H-6), 7.63 (s, H-2), 8.18 (d, J = 9 Hz, H-5). ^{1}H NMR (C_6D_6): δ 0.97, 1.06, 1.22 (t, each J = 7.1 Hz, all 3H, 3 × C$\underline{H}_3$ of EtO), 1.48 (br s, 3H, sidechain Me), 1.55 (d, J = 0.74 Hz, 3H, sidechain Me), 3.35 (q, J = 7.1 Hz, 2H, C$\underline{H}_2$ of EtO), 3.55 (br d − like, J = *ca* 7 Hz, 2H, 1″-C$\underline{H}_2$), 3.74 (q, J = 7.1 Hz, 4H, 2 × C$\underline{H}_2$ of EtO), 3.80 (s, 3H, OMe), 5.38 (br t, J = *ca* 7 Hz, 2″-C$\underline{H}$), 6.40 (s, H-3′), 6.56 (d, J = 2.2 Hz, H-8), 6.68 (dd, J = 8.8 and 2.2 Hz, H-6), 7.44 (s, H-2), 8.43 (d, J = 8.8 Hz, H-5).

*Kwakhurin epoxide (**14**)*

A cool (5−10 °C) solution of *m*-chloroperoxybenzoic acid (40 mg) in *p*-dioxane (3 ml) was added over a period of 90 min to kwakhurin (30 mg) in *p*-dioxane (11 ml) and acetonitrile (1 ml). After stirring at 15 °C for 8 h, the reaction mixture was diluted with EtOAc (100 ml), washed successively with aqueous $NaHSO_3$, aqueous $NaHCO_3$, and a saturated solution of NaCl, and then dried over Na_2SO_4. Si gel TLC (CAEM, 15:5:5:1) of the concentrated EtOAc solution afforded 23.3 mg of **14** (R_F 0.35). UV: λmax, nm: MeOH 216 sh, 241, 249, 294, 306 sh; + NaOMe 252 sh, 256, 302 sh, 333; + NaOAc 251, 257 sh, 289, 333 (addition of solid H_3BO_3 regenerated the MeOH spectrum). EI-MS (rel. int.): $[M]^+$ 384 (100), *m/z* 369 (18), 352 (7), 351 (30), 327 (7), 314 (7), 313 (7), 312 (8), 311 (9), 309 (6), 299 (11), 298 (7), 297 (26), 283 (6), 281 (9), 253 (5), 217 (6), 203 (6), 149 (6), 138 (9), 137 (96), 123 (6). ^{1}H NMR (acetone-d_6): δ 1.04 (d, J = 1.2 Hz, 3H, sidechain Me), 1.10 (br s, 3H, sidechain Me), 2.4−3.0 (m, 3H, 1″-C$\underline{H}_2$ and 2″-C$\underline{H}$; *cf.* corresponding data for epoxyluteone tetraacetate, δ 2.62−3.04 m [8]), 3.77 (s, 3H, OMe), 6.45 (s, H-3′), 6.93 (d, J = 2.4 Hz, H-8), 6.99 (dd, J = 8.3 and 2.4 Hz, H-6), 7.96 (s, H-2), 8.02 (d, J = 8.3 Hz, H-5).

*Kwakhurin pterocarpan (**5**)*

Kwakhurin (13 mg) in tetrahydrofuran (1 ml) and dry EtOH (1 ml) was stirred at 40−50 °C with solid $NaBH_4$ (13 mg). Further batches of $NaBH_4$ (each 13 mg) were added after 30, 60 and 90 min, and stirring was then continued at 40 °C for 5 h. The reac-

tion mixture was diluted with acetone (2 ml) and concentrated *in vacuo* to near dryness. The residue was treated with chilled 2 N HCl (5 ml), followed by saturated aqueous NaCl (5 ml), and the pterocarpan was then extracted with EtOAc. Si gel TLC (CAEM, 15:5:5:1) of this extract afforded crude **5** (6.5 mg; R_F 0.77–0.88) which was subsequently purified (Si gel TLC in CM, 25:1) to give 4.6 mg of the desired compound (R_F 0.58–0.71). UV: λmax, nm: MeOH 210, 229 sh, 282 sh, 287, 295 sh; + NaOMe 212, 248, 298. EI-MS (rel. int.): $[M]^+$ 354 (100), *m/z* 339 (43), 311 (19), 299 (11), 298 (50), 297 (23), 284 (11), 283 (49), 251 (11), 213 (19), 149 (15), 147 (16), 123 (30). ^{1}H NMR (acetone-d_6): δ 1.73, 1.81 (s, both 3H, 4′- and 5′-Me), 3.3–3.6 (m, 2H, H-6a/H-6ax), 3.39 (br d, J = 7.1 Hz, 2H, 1′-C$\underline{H}_2$), 3.71 (s, 3H, OMe), 4.29 (m, H-6eq), 5.23 (br t, J = 6.8 Hz, 2′-C$\underline{H}$), 5.32 (br d, J = 5.6 Hz, H-11a), 6.26 (s, H-10), 6.39 (d, J = 2.2 Hz, H-4), 6.56 (dd, J = 8.3 and 2.2 Hz, H-2), 7.31 (d, J = 8.3 Hz, H-1). NOE experiment: irradiation of the OMe signal enhanced only the 2′-C$\underline{H}$ resonance.

*Kwakhurin pterocarpan diethyl ether (**7**)*

Kwakhurin pterocarpan (5.8 mg) was ethylated with diethyl sulphate (50 µl) as described for kwakhurin triethyl ether (**6**). Work up and Si gel TLC (HE, 7:3) gave 5.1 mg of **7** (R_F 0.75). UV: λmax, nm: MeOH 208, 226 sh, 282 sh, 287, 293 sh. EI-MS (rel. int.): $[M]^+$ 410 (100), *m/z* 396 (14), 395 (48), 367 (19), 355 (12), 354 (44), 353 (12), 339 (12), 325 (31), 151 (14), 149 (18), 123 (14). ^{1}H NMR ($CDCl_3$): δ 1.41, 1.43 (t, each J = 7 Hz, both 3H, 2 × C$\underline{H}_3$ of EtO), 1.72, 1.79 (br s, both 3H, 4′- and 5′-Me), 3.35 (br d, J = 6.1 Hz, 2H, 1′-C$\underline{H}_2$), 3.4–3.6 (m, 2H, H-6a/H-6ax), 3.76 (s, 3H, OMe), 4.01, 4.02 (q, J = 6.8 Hz, and 7.1 Hz respectively, both 2H, 2 × C$\underline{H}_2$ of EtO), 4.28 (d – like, J = 6.4 Hz, H-6eq), 5.18 (br t, J = *ca* 6 Hz, 2′-C$\underline{H}$), 5.38 (d, J = 4.9 Hz, H-11a), 6.37 (s, H-10), 6.47 (d, J = 2.4 Hz, H-4), 6.62 (dd, J = 8.4 and 2.6 Hz, H-2), 7.39 (d, J = 8.3 Hz, H-1). ^{1}H NMR (C_6D_6): δ 1.05, 1.07 (t, each J = 7 Hz, both 3H, 2 × C$\underline{H}_3$ of EtO), 1.61, 1.68 (br s, both 3H, 4′- and 5′-Me), 3.2–3.7 (m, 2H, H-6a/H-6ax), 3.36 (br d, J = 5.4 Hz, 2H, 1′-C$\underline{H}_2$), 3.55, 3.56 (q, each J = 7 Hz, both 2H, 2 × C$\underline{H}_2$ of EtO), 3.75 (s, 3H, OMe), 4.29 (dd – like, J = 10.6 and 5 Hz, H-6eq), 5.26 (d – like, J = 6.3 Hz, H-11a), approx. 5.30 (2′-C$\underline{H}$; overlapping with H-11a signal), 6.45 (s, H-10), 6.66 (dd partly overlapped with signal at δ 6.71, J = 7.1 and 2.4 Hz, H-2), approx. 6.71 (incomplete d, H-4), 7.49 (d, J = 9.52 Hz, H-1).

*Kwakhurin coumaronochromone (**8**)*

Kwakhurin (12 mg) was warmed (approx. 50 °C for 30 min) with KF (9.7 mg) and dimethylformamide (0.5 ml) in a stoppered tube before being heated at 120 °C for 2 h. The reaction mixture was then diluted with EtOAc (50 ml), and after successive washing with dilute HCl/aqueous NaCl, aqueous $NaHCO_3$, and aqueous NaCl (equal volumes, all 0.5 N), the EtOAc layer was concentrated *in vacuo* and chromatographed (Si gel TLC in CAEM, 15:5:5:1) to afford unchanged starting material (6 mg) and 1.4 mg of **8** (R_F 0.78). UV: λmax, nm: MeOH 224 sh, 252 sh, 260, 286, 294 sh, 315 sh; + NaOMe 272, 307 (broad), 344; + NaOAc 259, 282 sh, 293 (broad), 335 (addition of solid H_3BO_3 regenerated the MeOH spectrum). EI-MS (rel. int.): $[M]^+$ 366 (100), *m/z* 351 (25), 335 (14), 333 (8), 323 (13), 312 (9), 311 (44), 310 (17), 309 (22), 298 (15), 297 (24), 296 (8), 283 (34), 281 (9), 255 (12), 215 (9), 168 (26), 137 (23). ^{1}H NMR (acetone-d_6): δ 1.63 (d, J = 1.2 Hz, 3H, sidechain Me), 1.85 (s, 3H, sidechain Me), 3.81 (s, 3H, OMe), 4.26 (br d, J = 6.6 Hz, 2H, 1″-C$\underline{H}_2$), 5.31 (br t, J = *ca* 6.8 Hz, 2″-C$\underline{H}$), 7.04 (s, H-3′), 7.06 (d, J = 2.2 Hz, H-8), 7.07 (dd, J = 9.2 and 2.2 Hz, H-6), 8.17 (d, J = 9.2 Hz, H-5). Lupinalbin A, a coumaronochromone from *Lupinus albus* roots [13], has also been recently prepared by heating 2′-hydroxygenistein (5,7,2′,4′-tetrahydroxyisoflavone) with KF (or CeF) and dimethylformamide as described above [12].

Formation of kwakhurin addition products

a) Reaction with formic acid

Kwakhurin (10 mg) was heated with 99% HCOOH (1 ml) at 80 °C for 6 h in a stoppered tube. After dilution with benzene (approx. 5 ml) and acetone (approx. 1 ml), the reaction mixture was reduced to dryness *in vacuo*. Si gel TLC of the residue (CAEM, 50:15:15:2, ×3) afforded unchanged kwakhurin (upper zone), a formic acid addition product (**10**, middle zone but near the starting material, 6.2 mg) and kwakhurin hydrate (**9**, lower zone, 0.6 mg). After elution, compounds **9** and **10** were further chromatographed in PEA (10:10:1, ×2). In

a second experiment, kwakhurin (4.5 mg in 50 µl of acetone) was heated (60 °C for 2 h, then 80 °C for 1 h) with 88% HCOOH (1 ml) to afford, after work up and Si gel TLC, approx. 1.8 mg of **9** and 1.1 mg of **10**.

*Kwakhurin hydrate (**9**)*

UV: λmax, nm: MeOH 216 sh, 241, 249, 292, 306 sh; + NaOMe 250 sh, 256, 298 sh, 332; + NaOAc 251, 257 sh, 289, 335 (addition of solid H_3BO_3 regenerated the MeOH spectrum). EI-MS (rel. int.): $[M]^+$ 386 (15), *m/z* 369 (6), 368 (M^+-H_2O; 27), 353 (8), 313 (6), 312 (11), 297 (17), 258 (6), 243 (10), 232 (11), 231 (5), 217 (5), 203 (11), 147 (6), 138 (8), 137 (100). ^{1}H NMR (acetone-d_6): δ 1.01 (s, 6H, 4″- and 5″-Me), 1.4–1.7 (m, 2H, 2″-C$\underline{H}_2$), 2.4–2.7 (m, 2H, 1″-C$\underline{H}_2$), 3.76 (s, 3H, OMe), 6.37 (s, H-3′), 6.91 (d, J = 2.2 Hz, H-8), 6.98 (dd, J = 8.6 and 2.2 Hz, H-6), 7.92 (s, H-2), 8.01 (br d, J = 8.6 Hz, H-5).

*Kwakhurin – formic acid addition product (**10**)*

UV: λmax, nm: MeOH 214 sh, 241, 249, 293, 305 sh; + NaOMe 256, 298, 333; + NaOAc 252–254 (broad), 290, 335 (addition of solid H_3BO_3 regenerated the MeOH spectrum). FD-MS (rel. int.): $[M]^+$ 414 (100), *m/z* 369 (37), 368 (M^+–HCOOH; 95). EI-MS (rel. int.): $[M]^+$ 414 (0.3), *m/z* 398 (0.9), 368 (M^+–HCOOH; 50), 352 (15), 312 (21), 297 (28), 281 (14), 203 (23), 137 (100). ^{1}H NMR (acetone-d_6): δ 1.32, 1.34 (s, both 3H, 4″- and 5″-Me), 1.8–2.0 (m, 2H, 2″-C$\underline{H}_2$), 2.3–2.7 (m, 2H, 1″-C$\underline{H}_2$), 3.76 (s, 3H, OMe), 6.39 (s, H-3′), 6.92 (d, J = 2.4 Hz, H-8), 6.99 (dd, J = 8.5 and 2.4 Hz, H-6), 7.85 (s, 3″-OCO$\underline{H}$), 7.93 (s, H-2), 8.02 (d, J = 8.5 Hz, H-5).

b) Reaction with HCl

Kwakhurin (20 mg) + acetonitrile (0.5 ml) and $AlCl_3$ (0.3 g) + acetonitrile (2.5 ml) were heated together (80 °C for 7 h) in a stoppered tube. Dilute HCl/aqueous NaCl (1:1, 10 ml, both 1 N) was then added and the mixture was shaken with EtOAc (50 ml). After washing with equal volumes of aqueous $NaHCO_3$ followed by aqueous NaCl (both 1 N), the EtOAc extract was reduced to dryness. Si gel TLC (CAEM, 15:5:5:1) of the residue gave cyclo-demethylkwakhurin (**12**, 2.6 mg) and a kwakhurin – HCl addition product (**13**, 16.3 mg) at R_F 0.61 and 0.54 respectively.

*Cyclo-demethylkwakhurin (**12**)*

UV: λmax, nm: MeOH 241, 249, 299, 305 sh; + NaOMe 255 (broad), 303 sh, 333 (broad); + NaOAc 251–255 (broad), 302 sh, 336 (broad) (addition of solid H_3BO_3 regenerated the MeOH spectrum). EI-MS (rel. int.): $[M]^+$ 354 (45), *m/z* 311 (13), 299 (7), 298 (26), 218 (7), 189 (9), 138 (9), 137 (100). ^{1}H NMR (acetone-d_6): δ 1.29 (s, 6H, 4″- and 5″-Me), 1.75 (t – like, J = 6.6 Hz, 2H, 2″-C$\underline{H}_2$), 2.53 (t – like, J = 6.8 Hz, 2H, 1″-C$\underline{H}_2$), 6.34 (s, H-3′), 6.92 (incomplete d, H-8), 6.99 (dd, J = 8.5 and 2.4 Hz, H-6), 7.95 (s, H-2), 8.03 (d, J = 8.5 Hz, H-5).

*Kwakhurin – hydrochloric acid addition product (**13**)*

UV: λmax, nm: MeOH 216 sh, 241, 249, 293 (broad), 306 sh; + NaOMe 256 (broad), 300 (broad), 332 (broad); + NaOAc 252, 255 sh, 289, 335 (broad) (addition of solid H_3BO_3 regenerated the MeOH spectrum). FD-MS (rel. int.): $[M]^+$ 404 (5), *m/z* 368 (M^+–HCl; 100). EI-MS (rel. int.): $[M]^+$ at *m/z* 404 not observed, *m/z* 369 (8), 368 (32), 353 (5), 312 (6), 297 (8), 258 (5), 243 (8), 232 (14), 231 (7), 217 (6), 203 (8), 169 (10), 138 (9), 137 (100). ^{1}H NMR (acetone-d_6): δ 1.43, 1.45 (s, both 3H, 4″- and 5″-Me), 1.8–2.0 (m, 2H, 2″-C$\underline{H}_2$), 2.5–2.8 (m, 2H, 1″-C$\underline{H}_2$), 3.77 (s, 3H, OMe), 6.41 (s, H-3′), 6.91 (incomplete d, H-8), 6.98 (dd, J = 8.4 and 2.2 Hz, H-6), 7.95 (s, H-2), 8.02 (br d, J = 8.4 Hz, H-5).

c) Reaction with acetic acid/conc. H_2SO_4

Kwakhurin (4.9 mg) was heated 60 °C; 15 min) with glacial HOAc (2.4 ml) and conc. H_2SO_4 (0.8 ml) as described for piscidone [11]. The reaction mixture was then poured into ice-cold aqueous NaCl and shaken with EtOAc. After being washed successively with aqueous $NaHCO_3$ and aqueous NaCl, the EtOAc extract was chromatographed (Si gel TLC in CAEM, 15:5:5:1) to give cyclo-demethylkwakhurin diacetate (0.4 mg, R_F 0.63–0.68), cyclo-demethylkwakhurin (**12**, 0.8 mg, R_F 0.39–0.45), a kwakhurin – acetic acid addition product (**11**, 0.3 mg, R_F 0.23–0.27), and kwakhurin hydrate (**9**, 0.6 mg, R_F 0.08–0.12). All four compounds were eluted and further purified by Si gel TLC in PEA (10:10:1). When kwakhurin (3 mg) was heated (5 min) at 60 °C with glacial HOAc (1.5 ml) and conc. H_2SO_4 (1 drop), and then kept at room temp. (approx.

18 °C) for 14 h, the reaction mixture yielded kwakhurin hydrate (0.8 mg) as essentially the only product.

Cyclo-demethylkwakhurin diacetate

EI-MS (rel. int.): $[M]^+$ 438 (5), *m/z* 397 (8), 396 (M^+-Ac; 32), 355 (12), 354 ($M^+-2\times Ac$; 50), 337 (8), 312 (8), 299 (10), 298 (37), 244 (7), 218 (10), 179 (7), 137 (100).

*Kwakhurin – acetic acid addition product (**11**)*

UV: λmax, nm: MeOH 214 sh, 241, 249, 293, 305 sh; + NaOMe 256 (broad), 298 sh, 334; + NaOAc 252, 257 sh, 288, 336 (addition of solid H_3BO_3 regenerated the MeOH spectrum). FD-MS (rel. int.): $[M]^+$ 428 (100), *m/z* 368 (M^+-HOAc; 24). EI-MS (rel. int.): $[M]^+$ 428 (1), *m/z* 368 (M^+-HOAc; 27), 313 (10), 312 (11), 297 (14), 281 (9), 203 (10), 137 (65). The lack of a fragment at M^+-42 (*cf.* preceding diacetate) in either the FD- or EI-MS of **11** indicated that none of the aromatic OH groups had been acetylated.

*Preparation of tetramethyl-licoisoflavone A (**15**)*

Licoisoflavone A (11.9 mg) from *Lupinus albus* roots [27] was methylated with dimethyl sulphate (200 µl) as described for kwakhurin trimethyl ether (**3**). Work up and Si gel TLC (BE, 7:3) gave 11.5 mg of **15** (R_F 0.49). UV: λmax, nm: MeOH 223 sh, 248 sh, 255, 280 sh, 308 sh. EI-MS (rel. int.): $[M]^+$ 410 (100), *m/z* 395 (28), 380 (25), 379 (95), 377 (8), 365 (9), 355 (17), 341 (25), 181 (27), 167 (8), 147 (9). ^1H NMR (acetone-d_6): δ 1.65, 1.76 (s, both, 3H, 4″- and 5″-Me), 3.36 (br d, J = 6.8 Hz, 2H, 1″-C$\underline{H}_2$), 3.51 (s, 3H, 2′-OMe), 3.86, 3.87 (s, both 3H, 5- and 4′-OMe), 3.94 (s, 3H, 7-OMe), 5.21 (br t, J = 6.8 Hz, 2″-C$\underline{H}$), 6.49 (d, J = 2.3 Hz, H-6), 6.59 (d, J = 2.3 Hz, H-8), 6.77 (d, J = 8.5 Hz, H-5′), 7.13 (d, J = 8.5 Hz, H-6′), 7.89 (s, H-2).

Reaction of tetramethyl-licoisoflavone A with formic acid

Tetramethyl-licoisoflavone A (6.3 mg) was heated (80 °C for 4 h) with 88% HCOOH (0.5 ml) in a stoppered tube. Work up as described for the reaction of kwakhurin with formic acid, and Si gel TLC (BE, 7:3) of the residue, gave a formic acid addition product (**16**, 1.9 mg) and a hydrate derivative (**17**, 3.5 mg) at R_F 0.26 and 0.05 respectively.

*Tetramethyl-licoisoflavone A – formic acid addition product (**16**)*

UV: λmax, nm: MeOH 224 sh, 248 sh, 255, 280 sh, 308 sh. FD-MS (rel. int.): $[M]^+$ 456 (100), *m/z* 410 ($M^+-HCOOH$; 2). EI-MS (rel. int.): $[M]^+$ 456 (3), *m/z* 440 (1), 425 (6), 411 (18), 410 ($M^+-HCOOH$; 62), 380 (27), 379 (100), 355 (60), 327 (20), 325 (22), 193 (29), 181 (22), 175 (18), 162 (17), 147 (18). ^1H NMR (acetone-d_6): δ 1.56 (s, 6H, 4″- and 5″-Me), 1.9–2.1 (m, 2H, 2″-C$\underline{H}_2$), 2.65–2.85 (m, 2H, 1″-C$\underline{H}_2$), 3.54 (s, 3H, 2′-OMe), 3.87, 3.88 (s, both 3H, 5- and 4′-OMe), 3.95 (s, 3H, 7-OMe), 6.50 (d, J = 2.4 Hz, H-6), 6.60 (d, J = 2.4 Hz, H-8), 6.77 (d, J = 8.5 Hz, H-5′), 7.13 (d, J = 8.5 Hz, H-6′), 7.91 (s, H-2), 8.17 (s, 3″-OCO$\underline{H}$).

*Tetramethyl-licoisoflavone A hydrate (**17**)*

UV: λmax, nm: MeOH 223 sh, 248 sh, 255, 280 sh, 308 sh. EI-MS (rel. int.): $[M]^+$ 428 (1), *m/z* 411 (20), 410 (M^+-H_2O; 69), 397 (28), 396 (26), 380 (27), 379 (100), 356 (16), 355 (68), 341 (29), 327 (20), 325 (24), 193 (30), 181 (30), 175 (18), 162 (22), 147 (22). ^1H NMR (acetone-d_6): δ 1.25 (s, 6H, 4″- and 5″-Me), 1.60–1.80 (m, 2H, 2″-C$\underline{H}_2$), 2.70–2.90 (m, 2H, 1″-C$\underline{H}_2$), 3.55 (s, 3H, 2′-OMe), 3.86, 3.88 (s, both 3H, 5- and 4′-OMe), 3.94 (s, 3H, 7-OMe), 6.49 (d, J = 2.3 Hz, H-6), 6.59 (d, J = 2.3 Hz, H-8), 6.76 (d, J = 8.5 Hz, H-5′), 7.11 (d, J = 8.5 Hz, H-6′), 7.89 (s, H-2).

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