Aureol and Phaseol, Two New Coumestans from *Phaseolus aureus* Roxb.

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Two new coumestans, aureol and phaseol, isolated from *Phaseolus aureus* have been characterized as 1,3,9-trihydroxycoumestan and 3,9-dihydroxy-4-(γ,γ-dimethylallyl)-coumestan, respectively.

In addition to the isoflavonoid phytoalexins reported earlier [1], the mung bean, *Phaseolus aureus*, produces coumestrol (**1**) and two novel coumestans, aureol (**2**) and phaseol (**3**). Coumestrol has been isolated from this plant previously [2] and the present communication describes the isolation and structural characterization of the latter two compounds.

The characterizations of aureol and phaseol as coumestans were initially suggested by their UV spectral properties. Coumestans generally exhibit high intensity absorption maxima in three wavelength regions. The position and relative intensity of the maximum in the mid-wavelength region gives some indication of substitution patterns in the aromatic B ring [3–6]. The UV spectrum of aureol possessed a mid-wavelength maximum at 257 nm which was of greater relative intensity than that at 337 nm, a feature so far known only in 3,7,9-trisubstituted coumestans [3–6]. However, 257 nm is a shorter wavelength than that of the corresponding peak in the latter compounds. Bathochromic shifts in the maximum of the UV spectrum of aureol upon addition of NaOAc and NaOMe indicated a free OH at C-3 together with one or more other phenolic functions. The formation of a triacetate established that aureol possessed three phenolic OH groups. The MS contained a plausible M^+ at *m/e* 284 with important fragment ions at *m/e* 256, 228 and 200, consistent with the behaviour of coumestans in which the only significant fragmentation is loss of CO moieties from the molecular ion [3].

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The PMR spectrum of aureol showed signals for 5 aromatic protons, one of which is *ortho* coupled, three are *meta* coupled and one is both *ortho* and *meta* coupled. Two possible structures can account for these features: 1,3,9-trihydroxycoumestan (**2**) and 3,7,9-trihydroxycoumestan (**4**). The downfield *ortho* coupled signal at δ7.81 could be attributed to the proton at C-7 or C-1. The *meta* coupled signal at δ7.14 can be assigned to a proton at either C-10 or C-4. The proton which resonates at δ7.02 and exhibits *ortho* and *meta* coupling may be located at either C-8 or C-2. The two remaining *meta* coupled signals at δ6.59 and δ6.37 may be assigned respectively to protons at C-4 or C-10 and C-2 or C-8. 3,7,9-Trihydroxycoumestan, "repensol", has been isolated previously from *Trifolium repens* [5]. Since both the UV spectral properties and TLC behaviour of aureol and its triacetate differ markedly from those of repensol and its triacetate, aureol is characterized as 1,3,9-trihydroxycoumestan.

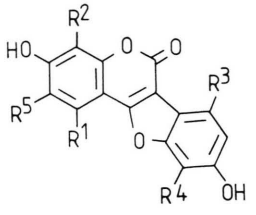
The UV spectrum of phaseol contains maxima at 210 nm, 250 nm and 343 nm, the mid-wavelength peak being of lower intensity than that at 343 nm. The spectrum undergoes a bathochromic shift upon addition of NaOAc and NaOMe indicating a free OH at C-3 and one or more other free phenolic functions in the molecule. MS of phaseol gave a strong molecular ion peak at *m/e* 336 and the fragmentation pattern is similar to that observed for the prenylated coumestans psoralidin [8] and sojagol [8]. Prominent fragment ions at *m/e* 281 and *m/e* 280 could be formed respectively by loss of C_4H_7 and C_4H_8 radicals from the molecular ion and considered together with the lack of an intense M^+-15 signal supported the presence of a γ,γ-dimethylallyl chain rather than a 2,2-dimethylchromene ring [9].

Remaining fragment ions in the MS were relatively minor. Signals at *m/e* 253 and 252 could result from loss of CO or CHO from the ions at *m/e* 281 and *m/e* 280.

The PMR spectrum confirmed that phaseol possessed a γ,γ-dimethylallyl unit and showed the presence of five aromatic protons, three of which show *ortho* coupling, one shows *meta* coupling and the remaining proton is both *ortho* and *meta* coupled. Two possible structures can account for the spectral features of phaseol: 3,9-dihydroxy-10(γ,γ-dimethylallyl)-coumestan (**5**) and 3,9-dihydroxy-4(γ,γ-dimethylallyl)-coumestan (**3**). The two downfield doublet signals at δ7.94 and δ7.63, both of which are *ortho* coupled, can be attributed to protons at C-1 and C-7. Another aromatic proton resonating at δ7.07 and showing *ortho* coupling could be located either at C-2 or C-8. The signal at δ6.97 is *meta* coupled and can be attributed to the proton at C-4 or C-10. The remaining aromatic proton resonating at δ7.04 exhibits *ortho* and *meta* coupling and may be located at C-8 or C-2.

Acid cyclisation of phaseol yielded a product whose UV spectrum did not undergo a bathochromic shift in NaOAc, but underwent a shift of 23 nm in NaOMe. Thus cyclisation apparently involves the C-3 OH rather than the C-9 OH. Phaseol is thus identified as 3,9-dihydroxy-4-(γ,γ-dimethylallyl)-coumestan. Phaseol is the second prenylated coumestan found to occur in the genus *Phaseolus*. Psoralidin (**6**) has been isolated previously from *P. lunatus* [10].

Phaseol co-occurs in *P. aureus* with the prenylated isoflavanone 5-deoxykievitone [1]. By analogy with the established biosynthesis of coumestrol from 4[1],7-dihydroxyisoflavanone presumably via 2[1],4[1],7-trihydroxyisoflavanone [2, 11, 12] it is conceivable that 5-deoxykievitone may be a biogenetic precursor of phaseol. This hypothetical pathway would be a contrast to the biosynthesis of the prenylated pterocarpans phaseollidin and phaseollin [13, 14] and the glyceollins [15] in which prenylation appears to occur after the formation of the pterocarpan nucleus.



1. $R^1 = R^2 = R^3 = R^4 = R^5 = H$
2. $R^1 = OH$, $R^2 = R^3 = R^4 = R^5 = H$
3. $R^1 = R^3 = R^4 = R^5 = H$, $R^2 = CH_2\,CH{=}C(CH_3)_2$
4. $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = OH$
5. $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = CH_2\,CH{=}C(CH_3)_2$
6. $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = CH_2\,CH{=}C(CH_3)_2$

Experimental

Extraction procedure

P. aureus seedlings were grown and treated as previously described [1]. EtOH extracts were chromatographed on Si gel GF_{254} and developed in Hexane-EtOAc-MeOH (6-4-1) (Solvent A). Aureol and phaseol were further purified on TLC in Hexane-Acetone (2-1) (Solvent B) and $CHCl_3$OMeOH (10-1) (Solvent C).

Aureol (**2**)

Yield 14.9 µg/g fr. wt. Detected as a yellow fluorescent band on TLC at R_f 0.50, 0.30 and 0.60 in solvents A, B and C, respectively. UV EtOH λ_{max} nm: 212, 257, 284 sh, 303 sh, 337; EtOH + NaOAc: 212, 257, 292 sh, 345; EtOH + NaOMe: 220, 272, 305, 362; MS *m/e* (rel. intens.): 284 (100) $C_{15}H_{18}O_6$, M^+, 256 (6) M^+-CO, 255 (12) M^+-CHO, 228 (6) M^+-6, 200 (12); PMR 400 MHz $(CD_3)_2CO$: δ7.81 (1 H, d, $J = 8.3$ Hz, C-7), δ7.14 (1 H, d, $J = 1.9$ Hz, C-10), δ7.02 (1 H, d, d, $J = 8.4$ Hz, 2.1 Hz, C-8), δ6.59 (1 H, d, $J = 1.8$ Hz, C-4), δ6.37 (1 H, d, $J = 1.82$ Hz, C-2).

Acetate

Treatment of **2** with Ac_2O-pyridine gave a triacetate located as a blue fluorescent band at R_f 0.45 in solvent B; UV EtOH λ_{max} nm: 210, 245, 251, 256 sh, 264 sh, 274 sh, 310; MS *m/e* (rel. intens.): 410.0623 (2) M^+ (Calc for $C_{21}H_{14}O_9$ 410.0637), 368 (15) M^+-Ac, 326 (33) M^+-2 Ac, 284 (100) M^+-3 Ac.

Phaseol (**3**)

Yield 8.5 µg/g fr. wt. Detected as a blue fluorescent band on TLC at R_f 0.60, 0.43 and 0.45 in

solvents A, B and C, respectively. UV EtOH λ_{max} nm: 210, 250, 301, 343; EtOH + NaOAc: 215, 251, 308, 362; EtOH + NaOMe: 280, 383; MS *m/e* (rel. intens.): 336 (44) $C_{30}H_{26}O_5$, M^+, 321 (3), 319 (13), 281 (53) M^+-C_4H_7, 280 (100) M^+-C_4H_8, 253 (6), 252 (9); PMR 400 MHz, $(CD_3)_2CO$: δ7.94 (1H, d, $J = 8.7$ Hz, C-1 or C-7), δ7.63 (1H, d, $J = 8.5$ Hz, C-7 or C-1), δ7.07 (1H, d, $J = 8.5-8.7$ Hz, C-6), δ7.05 (1H, d, d, $J = 8.5-8.7$ Hz, 2.3 Hz, C-8), δ6.97 (1H, d, $J = 2.3$ Hz, C-10), δ5.46 (1H, brt., $J \cong 7.5$ Hz, C-2[1]), δ3.74 (2H, d, $J \cong 7.5$ Hz, C-1[1]), δ1.95 (3H, s, Me), δ1.71 (3H, s, Me). Acid cyclization of (3): phaseol (0.5 mg), MeOH (0.30 ml), cHCl (0.09 ml) were refluxed for $1\frac{1}{2}$ h. Cyclized phaseol: UV EtOH λ_{max} nm: 301, 343; EtOH + NaOAc: 301, 343; EtOH + NaOMe: 308, 366.

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