Phytochemistry, Vol. 25, No. 6, pp. 1315–1322, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

INDUCIBLE ISOFLAVONOIDS FROM THE LIMA BEAN, *PHASEOLUS LUNATUS*

MELANIE J. O'NEILL*, S. A. ADESANYA†, MARGARET F. ROBERTS and INEZ R. PANTRY

Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London, WC1N 1AX, U.K.

(*Received* 26 *September* 1985)

Key Word Index—*Phaseolus lunatus*; Leguminosae; isoflavonoid; phytoalexin; antifungal.

Abstract—*Phaseolus lunatus* seedlings treated with aqueous cuprous chloride produced 25 isoflavonoids which were isolated and characterized by UV, mass and ^{1}H NMR spectroscopy as kievitone, isoferreirin, 5-deoxykievitone, kievitone hydrate, cyclokievitone, cyclokievitone hydrate, daidzein, 2′-hydroxydaidzein, genistein, 2′-hydroxygenistein, 2,3-dehydrokievitone, luteone, phaseollidin, 2-(γ,γ-dimethylallyl)-phaseollidin, coumestrol, psoralidin, 7,8,2′,4′-tetrahydroxyisoflavone, 5,7,8,2′,4′-penta hydroxyisoflavone, 2,3-dehydrokievitol, lunatone, 5-deoxykievitol, kievitol, 3′-(γ,γ-dimethylallyl)-kievitone, 4-(γ,γ-dimethylallyl)-phaseollidin and 2-(γ,γ-dimethylallyl)-6a-hydroxy-phaseollidin. The last nine compounds have novel structures. Possible biogenetic routes for these isoflavonoids and the taxonomic implications of their occurrence are discussed.

INTRODUCTION

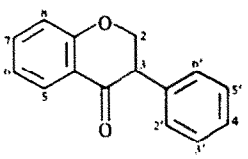
In common with other papilionate legumes, members of the genus *Phaseolus* examined to date [1–4], produce isoflavonoids as phytoalexins in response to stress. Some of these compounds possess a wide spectrum of activity against phyto- and zoo-pathogenic fungi [5, 6]. In our continuing study for novel, potentially valuable antifungal agents we have examined the inducible isoflavonoids produced by the lima bean, *P. lunatus* L. *Phaseolus lunatus* is essentially a food crop, the mature beans being a valuable pulse especially in tropical Africa. Large quantities of lima beans are consumed in the U.S.A. as a vegetable [7]. The plant has also a reputation in folk medicine: the seeds have been used a diet food in fevers, decoctions of green pods, seeds and stems have been used to treat diabetes and dropsy and in Java, poultices made from the seeds are used on the abdomen for stomach ache [8]. Antifungal activity has been demonstrated in ethanolic extracts of *P. lunatus* cotyledons infected with *Phytophthora vignae* [9] and activity against the nematode *Pratylenchus scribneri* has been shown to be associated with the presence of the coumestans coumestrol and psoralidin in lima bean roots [10]. The present report describes the isolation of 25 isoflavonoids, including nine novel structures, from *P. lunatus* seedlings treated with cuprous chloride.

RESULTS AND DISCUSSION

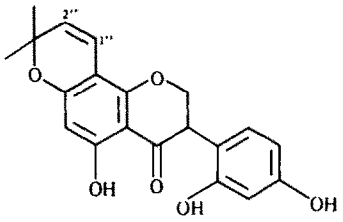
Ethanol extracts from *P. lunatus* seedlings treated with cuprous chloride were chromatographed over polyamide columns and silica gel thin layers and yielded a large number of isoflavonoids. By far the most abundant isoflavonoid in *P. lunatus* is the prenylated isoflavanone kievitone (**1**), which is known as a principal phytoalexin in other *Phaseolus* and *Vigna* species [1]. This compound co-occurs in *P. lunatus* with the isoflavanones isoferreirin (**2**), 5-deoxykievitone (**3**), kievitone hydrate (**4**), cyclokievitone (**5**) and cyclokievitone hydrate (**6**), all of which have previously been isolated from other *Phaseolus* species [2–4, 11]. Six isoflavones extracted from the plant were readily identified as daidzein (**7**), 2′-hydroxydaidzein (**8**), genistein (**9**), 2′-hydroxygenistein (**10**), 2,3-dehydrokievitone (**11**) and luteone (**12**) by a comparison of their spectral characteristics with authentic standards or literature values. The prenylated pterocarpans phaseollidin (**13**) and 2-(γ,γ-dimethylallyl)-phaseollidin (**14**) and the coumestans coumestrol (**15**) and psoralidin (**16**) were also detected in the plant. In addition to the above compounds nine novel isoflavonoids were isolated from *P. lunatus*. Four of these are novel isoflavones, the first of which (**17**) possesses a UV spectrum which is very similar to that of daidzein (**7**) [12], having a principal maximum at 249 nm. The shift in this maximum upon addition of sodium acetate and the further shift caused by addition of sodium methoxide indicated a 7-hydroxyl and one or more additional hydroxyl groups in the molecule [1]. A bathochromic shift in the methanolic UV maximum was also observed upon adding aluminium chloride, indicating the presence of a 5-hydroxyl and/or a 2′-hydroxyl group. This shift was almost abolished in the presence of HCl(aq.), suggesting the existence of an *ortho*-dihydroxyl moiety [13]. The isoflavone nature of the compound was confirmed by ^{1}H NMR spectroscopy, which showed the typical C-2 proton singlet at δ 8.39. The aromatic signals included an ABX system and two *ortho*-coupled doublets. The ABX system was attributed to B ring protons at C-6′ (δ 6.98), C-5′ (δ 6.45) and C-3′ (δ 6.47). These values are very similar to those for protons at C-6′ (δ 7.17), C-5′ (δ 6.43) and C-3′ (δ 6.47) in 2′-hydroxygenistein (**10**) [14].

*Author to whom correspondence should be addressed.

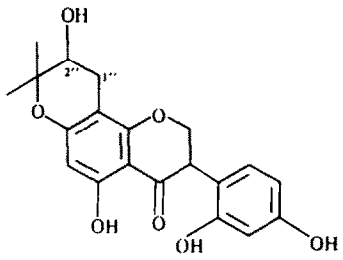
†Present address: Department of Pharmacognosy, Faculty of Pharmacy, University of Ife, Ile-Ife, Nigeria.



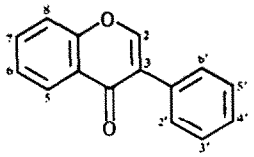
1 5 = 7 = 2′ = 4′ = OH, 8 = $CH_2CH{=}CMe_2$
2 5 = 7 = 4′ = OH, 2′ = OMe
3 7 = 2′ = 4′ = OH, 8 = $CH_2CH{=}CMe_2$
4 5 = 7 = 2′ = 4′ = OH, 8 = $CH_2CH_2CMe_2OH$
22 7 = 2′ = 4′ = OH, 8 = $CH_2CH{=}CMeCH_2OH$
23 5 = 7 = 2′ = 4′ = OH, 8 = $CH_2CH{=}CMeCH_2OH$
24 5 = 7 = 2′ = 4′ = OH, 8 = 3′ = $CH_2CH{=}CMe_2$
25 5 = 7 = 2′ = 4′ = OH, 6 = 3′ = $CH_2CH{=}CMe_2$
26 5 = 7 = 4′ = OH, 6 = 3′ = $CH_2CH{=}CMe_2$, 2′ = OMe



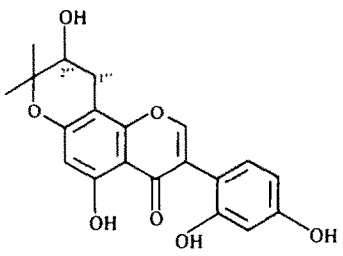
5



6



7 7 = 4′ = OH
8 7 = 2′ = 4′ = OH
9 5 = 7 = 4′ = OH
10 5 = 7 = 2′ = 4′ = OH
11 5 = 7 = 2′ = 4′ = OH, 8 = $CH_2CH{=}CMe_2$
12 5 = 7 = 2′ = 4′ = OH, 6 = $CH_2CH{=}CMe_2$
17 7 = 8 = 2′ = 4′ = OH
18 5 = 7 = 8 = 2′ = 4′ = OH
19 5 = 6 = 7 = 4′ = OH
20 5 = 7 = 2′ = 4′ = OH, 8 = $CH_2CH{=}CMeCH_2OH$



21

The *ortho*-coupled doublets at δ8.06 and 7.12 were attributed to the C-5 and C-6 protons respectively. The compound gave a poor EIMS, possibly because of intermolecular H-bonding involving the *ortho*-hydroxyl groups, and low yields prevented derivatization. The available spectroscopic data imply the structure of **17** to be 7,8,2′,4′-tetrahydroxyisoflavone.

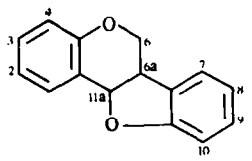
The second novel isoflavone isolated (**18**) was a 5-hydroxy analogue of the previous compound. The methanolic UV spectrum contained a principal maximum at 265 nm which underwent shifts in the presence of sodium acetate, sodium methoxide and aluminium chloride, indicating the presence of 7-hydroxyl and additional hydroxyl groups including one at C-2′ and/or C-5. A reduction in the aluminium chloride shift brought about by addition of HCl (aq.) implied the existence of an *ortho*-dihydroxyl moiety. The ^{1}H NMR spectrum exhibited the isoflavone C-2 proton singlet at δ8.25 and an aromatic ABX system which is almost identical to that arising from the B ring protons of 2′-hydroxygenistein (**10**) and **17** and so was attributed to protons at C-6′ (δ7.15), C-5′ (δ6.44) and C-3′ (δ6.49) in the new compound. The only remaining signal in the ^{1}H NMR spectrum at δ6.38 is due to a single uncoupled proton. This differs slightly from the C-8 proton (δ6.32) of 6-hydroxygenistein (**19**) [15]. In an NOE experiment, irradiation at the C-5 hydroxyl proton singlet at δ12.65 produced a small enhancement of the δ6.38 signal. This latter signal was thus assigned to the proton at C-6. The compound did not produce a useful EIMS and lack of material prevented derivatization. The spectroscopic evi-

dence so far suggests the structure of **18** to be 5,7,8,2',4'-pentahydroxyisoflavone.

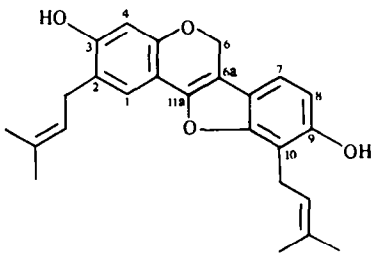
The third new isoflavone (**20**) obtained from *P. lunatus* possesses a UV principal absorption maximum at 265 nm. Its ^{1}H NMR spectrum was almost identical to that of 2,3-dehydrokievitone (**11**) [11], having a 1H singlet (C-2) at δ8.25, a 1H singlet (C-6) at δ6.36 and an ABX system of aromatic signals at δ7.13 (C-6'), 6.45 (C-5') and 6.49 (C-3'). The remaining signals which were attributed to a substituent at C-8 were similar to those of a γ,γ-dimethylallyl side chain, having a 1H broad triplet at δ5.34 (the vinylic C-2" proton) and a broad 2H doublet at δ3.56 (the methylene protons at C-1"). However the spectrum contained only one 3H singlet at δ1.74 indicating the presence of only one methyl group on the isopentenyl chain. The absent second methyl signal is replaced by a 2H singlet at δ4.32 in the new compound and this is typical of the resonance frequency for protons α to an alcoholic group. The presence of an extra oxygen in the new molecule was confirmed by EIMS which shows an $[M]^+$ at m/z 370 (27%) compared to the $[M]^+$ at m/z 354 for 2,3-dehydrokievitone. The $[M]^+$ at m/z 370 readily loses oxygen, OH and H_2O to give peaks at m/z 354 (35%), 353 (100%) and 352 (93%) respectively. The A- and B-ring fragments after RDA, at m/z 219 (64%) and 314 (84%) respectively confirm that the side chain is on the A ring of the molecule. The presence of the extra oxygen on the side chain is indicated by peaks at m/z 337 (87%), 311 (45%) and 299 (72%) representing $[M-MeOH]^+$, $[M-C_3H_6OH]^+$ and $[M-C_4H_6OH]^+$ respectively. Thus in **20**, one methyl group of the isopentenyl side chain has been oxidized to a primary alcohol. Compound **20** has thus been characterized as 5,7,2',4'-tetrahydroxy-8-(4"-hydroxy-3"-methyl-but-2"-enyl)-isoflavone, for which we propose the trivial name 2,3-dehydrokievitol. This type of alcoholic side chain has only been found previously in the pterocarpan carbenegrins A–I which have been isolated from an unidentified South American legume [16].

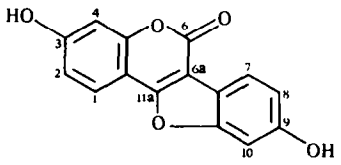
The final isoflavone isolated (**21**) gave a UV maximum at 266 nm which did not shift upon addition of sodium acetate, indicating that the C-7 hydroxyl is derivatized or absent. Shifts observed upon adding aluminium chloride indicated a C-5 OH and/or a C-2' OH. EIMS revealed that **21**, having an $[M]^+$ at m/z 370 (100%), was an isomer of 2,3-dehydrokievitol (**20**). The $[M]^+$ of **21** also dehydrates to give the peak at m/z 352 (22%) but this transition is less easy than for **20**. In the ^{1}H NMR spectrum of **21**, the C-2 proton resonates at δ8.16 and the ABX system of aromatic protons was assigned to the C-6' (δ7.11), C-5' (δ6.34) and C-3' (δ6.45) on the basis of their close similarity to corresponding protons in 2,3-dehydrokievitone. The C-6 proton in **21** resonates at δ6.24 which is slightly further upfield than the signal for the same proton (δ6.42) in 2,3-dehydrokievitone, as expected from the UV evidence that C-7 does not bear a free hydroxyl. The remaining ^{1}H NMR signals, two 3H singlets at δ1.26 and 1.30, a 2H double doublet at δ3.30 and a 1H triplet at δ4.88, suggested that the prenyl moiety in **21** is analogous to that observed in the isoflavanone cyclokievitone hydrate (**6**) which we have also isolated from this plant and earlier from *P. mungo* [3].

In the latter compound, the prenyl group at C-8 is cyclised with the C-7 hydroxyl to give the dimethylchroman system which is substituted by OH either at C-1" or C-2". The existence of this hydroxylated dimethylchroman system in **21** was supported by the presence in the EIMS of peaks at m/z 221 (8%) and 203 (21%). These are attributable to the RDA A-ring fragments **21a** and **21b** before and after dehydration across the C-1"/C-2" bond in the $[M]^+$. The location of the hydroxyl group at either the benzylic carbon C-1" or the vicinal position C-2" in the chroman system was established by NOE experiments in the ^{1}H NMR. Thus irradiation at either of the two methyl signals of the chroman unit produced enhancement of both the 2H double doublet at δ3.30 and the 1H triplet at δ4.88. The latter signal experienced greater enhancement than the former, whichever of the two methyl signals was irradiated. Since it is known [17] that in general NOE decreases with increasing internuclear distance, in the present case the single proton resonating at δ4.88 should be nearer to the methyl groups than the two

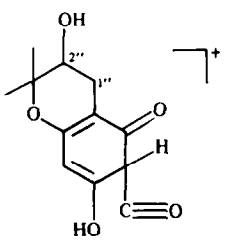


13 3 = 9 = OH, 10 = $CH_2CH{=}CMe_2$
14 2 = 10 = $CH_2CH{=}CMe_2$, 3 = 9 = OH
27 3 = 9 = OH, 4 = 10 = $CH_2CH{=}CMe_2$
28 2 = 10 = $CH_2CH{=}C(Me)_2$, 3 = 6a = 9 = OH
29 3 = 6a = 9 = OH
30 3 = 6a = 9 = OH, 4 = 8 = $CH_2CH{=}CMe_2$

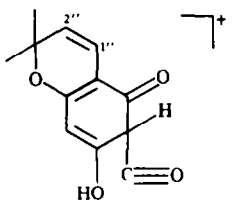




15
16 2 = $CH_2CH{=}CMe_2$



21a



21b

protons which resonate at δ3.30. Hence the hydroxyl group presumably resides at C-2″ as in **21**. Dreiding models show that a single proton at C-2″ is always closer to the methyls at C-3″ than could be a single proton at C-1″. The compound has been assigned the trivial name lunatone.

The first of the three novel isoflavanones isolated (**22**) was found to be a 5-deoxyisoflavanone since its UV spectrum contained a principal maximum at 286 nm which did not undergo a shift upon addition of aluminium chloride. The ^{1}H NMR spectrum contained the complex of signals for the C-2a, C-2b and C-3 protons of an isoflavanone [18] at δ4.70, δ4.61 and δ4.15 respectively. The aromatic signals were almost identical to those of 5-deoxykievitone (**3**) [11], having a pair of *ortho*-coupled signals at δ7.66 and 6.61 due to the C-5 and C-6 protons respectively, and an ABX system arising from the protons at C-6′ (δ6.98), C-5′ (δ6.35) and C-3′ (δ6.44). The nature of the substituent at C-8 was revealed to be identical to the oxidized isopentenyl chain found in 2,3-dehydrokievitol (**20**). In **22**, which we have named 5-deoxykievitol, the vinylic proton (C-2″) resonates as a triplet at δ4.70 and the methylene protons at C-1″ give rise to a doublet at δ3.45. The methyl group at C-3″ resonates as a singlet at δ1.76 and the two protons at C-4″, α to the alcoholic group produce a singlet at δ4.29. EIMS indicated a $[M]^+$ at *m/z* 356 (14%) which readily loses an oxygen atom and H_2O to give ions at *m/z* 340 (21%) and 338 (29%). The ions at *m/z* 221 (15%) and 136 (51%) represent the A and B ring fragments after RDA fragmentation of the $[M]^+$ and confirm that the oxidized prenyl chain is located on the A-ring. Compound **22** was therefore characterized as 7,2′,4′-trihydroxy-8-(4″-hydroxy-3″-methyl-but-2″-enyl)-isoflavanone.

The second novel isoflavanone (**23**) represented the third isoflavonoid, isolated from *P. lunatus*, possessing a prenyl side chain in which one of the terminal methyls has been oxidized to a primary alcohol. In the ^{1}H NMR spectrum, the signals for the C-2a, C-2b and C-3 protons occurred at δ4.64, 4.54 and 4.23 respectively. Signals for four aromatic protons at δ6.01 (C-6), 6.34 (C-5′), 6.96 (C-6′) and 6.44 (C-3′) were identical to those due to corresponding protons in kievitone [19, 20]. The broad triplet at δ5.30 due to the proton at C-2″ and the doublet at δ3.33 due to the methylene protons at C-1″ did not differ substantially from corresponding protons in kievitone. However the two singlets due to the two methyl groups at C-3″ in kievitone are now replaced in the spectrum of the new compound by one methyl group signal at δ1.73 and a 2H singlet at δ4.26. Thus the new compound (**23**) bears at C-3″ a methyl and a CH_2OH group. EIMS revealed an $[M]^+$ at *m/z* 372 (5%), 16 mass units higher than kievitone, which lost H_2O rapidly to give an ion at *m/z* 354 (50%). The base peak of the spectrum at *m/z* 219 is attributable to the A-ring fragment bearing an oxidized prenyl unit. Compound **23** was therefore characterized as 5,7,2′,4′-tetrahydroxy-8-(4″-hydroxy-3″-methyl-but-2″-enyl)-isoflavanone, for which we propose the trivial name kievitol.

The remaining new isoflavanone (**24**) possesses a UV absorption maximum at 295 nm which undergoes bathochromic shifts upon addition of sodium acetate, sodium methoxide or aluminium chloride indicating that at least 7- and 5-hydroxyl groups are present. The ^{1}H NMR spectrum shows the typical coupling of the C-2a, C-2b and C-3 protons of an isoflavanone at δ4.80, 4.68 and 4.00 respectively. There are three aromatic protons, two of which at δ6.42 and 7.09, are both *ortho*-coupled and the third, which resonated at δ5.99 is a singlet. Since 5- and 7-hydroxyl groups are present, the two *ortho*-coupled protons must be located on ring B and the singlet proton must reside on ring A. The ^{1}H NMR spectrum also indicated the presence of two γ,γ-dimethylallyl chains by signals at δ1.67, 1.77 (4 × Me), 3.23, 3.40 (2 × $-C\underline{H}_2CH$) and 5.27 (2 × $-CH_2-C\underline{H}=C$). EIMS revealed an $[M]^+$ at *m/z* 424 (51%) and RDA A- and B-ring fragments at *m/z* 221 (77%) and 204 (22%) respectively, indicating the presence of one isopentenyl chain on each of the A and B-rings. Two possible structures can account for the spectral features: 5,7,2′,4′-tetrahydroxy-8,3-di(γ,γ-dimethylallyl)-isoflavanone (**24**) and 5,7,2′,4′-tetrahydroxy-6,3′-di(γ,γ-dimethylallyl)-isoflavanone (**25**). Thus the isopentenyl chain on ring A may be either at C-8 as in kievitone (**1**) or at C-6 as in isosophoranone (**26**). A comparison of the resonance values for the C-6 proton of kievitone (δ5.98) [19, 20] and the C-8 proton in isosophoranone (δ5.75) [21, 22] reveals that the singlet aromatic proton in the new compound (δ5.99) is more like the C-6 proton of kievitone. This is supported by the ^{13}C NMR resonance values of the C-6 (δ95.20) and the C-8 (δ106.8) which are very close to the values for these carbons (C-6 δ94.8, C-8 δ106.4) reported for kievitone [23]. Confirmation of the structure of the new compound as **24** was provided by an inspection of literature values for UV shifts of prenylated isoflavanones upon addition of aluminium chloride [21]. Isosophoranone, along with other isoflavanones containing bulky prenyl groups *ortho* to a 5-hydroxyl, does not exhibit a shift in the UV maximum upon addition of aluminium chloride [22]. The new diprenylated isoflavanone (**24**) possesses a UV maximum which undergoes a bathochromic shift of 22 nm in aluminium chloride–methanol.

The two remaining novel isoflavonoids isolated from *P. lunatus* are both diprenylated pterocarpans. The first (**27**), was found to be an isomer of 2-(γ,γ-dimethylallyl)-phaseollidin (**14**), by EIMS which indicated an $[M]^+$ at *m/z* 392 (100%) and prominent peaks at *m/z* 336 (27%) and 280 (54%) representing loss of two C_4H_8 units from two isopentenyl side chains. The ^{1}H NMR spectrum showed the typical ABMX system for the C-6_{ax} (δ4.28), C-6_{eq} (δ3.60), C-6a (δ3.50) and C-11a (δ5.48) protons of a pterocarpan [18]. Also obvious were signals for two γ,γ-dimethylallyl chains at δ1.74 and 1.81 (4 × Me), 3.45 (2 × $-C\underline{H}_2CH$), 5.22 and 5.29 (2 × $-CH_2-C\underline{H}=C$) and signals for four aromatic protons, representing two *ortho*-coupled pairs at δ6.38 and 6.98 and at δ6.58 and 7.30. Two D_2O-exchangeable proton signals at δ5.29 and 5.35 were attributed to two phenolic moieties. In the UV spectrum, shifts in the maximum observed upon addition of sodium methoxide, together with the reaction of the compound with diazotized *p*-nitroaniline to produce an orange colour [1, 24] located the phenolic groups at C-3 and C-9. Thus both the A- and B-rings contain one pair of *ortho*-coupled protons and one γ,γ-dimethylallyl chain. The signals at δ6.38 and 6.98 were assigned to protons at C-8 and C-7 respectively as they are almost identical to the corresponding protons in phaseollidin at δ6.38 (C-8) and 6.95 (C-7), and in **14** at 6.38 (C-8) and 6.96 (C-7). The remaining pair of *ortho*-coupled signals at δ6.58 and 7.30 must arise from the protons at C-2 and C-1. The compound was thus identified as 4-(γ,γ-dimethylallyl)-phaseollidin (**27**).

The final novel compound (**28**) was identified initially as a 6a-hydroxypterocarpan by its dehydration in acid to form a pterocarpene [1]. EIMS of the pterocarpene showed an $[M]^+$ at m/z 390 (100%) which lost two C_4H_7 units, presumably from two isopentenyl groups, to give peaks at m/z 335 (26%) and 279 (39%). C-3 and C-9 dihydroxylation was established by shifts in the UV maximum upon addition of sodium methoxide and the reaction of the compound with diazotized *p*-nitroaniline to give an orange colour. In the ^{1}H NMR spectrum of the pterocarpan, a singlet at δ5.24 for the C-11a proton, and doublets at δ3.94 and 4.17 representing the C-6 protons are similar to the signals for corresponding protons in glycinol (**29**) [25] at δ5.26 (C-11a), 4.02 (C-6_{ax}) and 4.16 (C-6_{eq}) and confirm the C-6a hydroxylation of the new compound. The ^{1}H NMR spectrum contained signals for two γ,γ-dimethylallyl chains at δ1.74 (2 × Me), 1.76 (2 × Me), 3.34 (2 × -C$\underline{H}_2$CH), 5.30 (2 × -CH$_2$-C$\underline{H}$=C) and signals for four aromatic protons including two singlets at δ6.40 and 7.22 and two *ortho*-coupled protons at δ6.44 and 7.08. Two structures can account for these features: 3,6a,9-trihydroxy-2,10-di(γ,γ-dimethylallyl)-pterocarpan (**28**) or 3,6a,9-trihydroxy-4,8-di(γ,γ-dimethylallyl)-pterocarpan (**30**). The former structure is preferred since the ^{1}H NMR singlets at δ6.40 and 7.22 have almost identical shifts to those reported for the C-4 (δ6.41) and C-1 (δ7.25) protons respectively in **14**, whereas the doublets at δ6.44 and 7.08 in the new compound are quite different from those of the ring A protons of **27** at δ6.58 (C-2) and 7.30 (C-1). Structure **28** is also preferred on biosynthetic grounds: prenylation at C-8 (or the equivalent C-5′ of isoflavones and isoflavanones) is unknown in the genus *Phaseolus*. Compound **28** has been assigned the trivial name 2(γ,γ-dimethylallyl)-6a-hydroxyphaseollidin.

The occurrence of the nine novel isoflavonoids in *P. lunatus* can be rationalized on the basis of proposed metabolic grids for isoflavonoids in other *Phaseolus* species [4, 11, 26–28]. 2,3-Dehydrokievitol (**20**), kievitol (**23**) and 5-deoxykievitol (**22**) presumably arise by oxidation at terminal methyls of the dimethylallyl chains respectively in 2,3-dehydrokievitone (**11**), kievitone (**1**) and 5-deoxykievitone (**3**), all of which co-occur in the plant. The isoflavone lunatone (**21**) could be derived by cyclization of the prenyl chain in 2,3-dehydrokievitone to give a chromene followed by hydration to the chromanol. An analogous route in the isoflavanone series would generate cyclokievitone hydrate (**6**) from kievitone (**1**). 7,8-*ortho*-Dihydroxyisoflavonoids, **17** and **18**, have been encountered in the genus *Phaseolus* for the first time in *P. lunatus*. Radiotracer experiments in *Onobrychis viciifolia* [29] indicate that 6-hydroxy/methoxy substituents can be introduced after the formation of an isoflavone and thus the most likely path for the biogenesis of **17** and **18** would involve direct hydroxylation at C-8 either of 2′-hydroxydaidzein (**8**) to give **17** or of 2′-hydroxygenistein (**10**) to give **18**. Both of the proposed immediate precursors are available in the plant.

Phaseolus lunatus is perhaps most strikingly different from other *Phaseolus* species in that it produces diprenylated isoflavonoids. The plant apparently has a very active or indiscriminate dimethylallyl transferase system. 3′-(γ,γ-dimethylallyl)-Kievitone (**24**) and 4-(γ,γ-dimethylallyl)-phaseollidin (**27**) are probably derived by direct prenylation respectively at C-3′ of kievitone (**1**) and C-4 of phaseollidin (**13**). The prenylated 6a-hydroxypterocarpan (**28**) could arise alternatively from glycinol (**29**) by prenylation at C-4 and C-10 or by 6a-hydroxylation and C-4 prenylation of phaseollidin (**13**). In the biosynthesis of the glyceollins I, II and III in *Glycine max*, it has been demonstrated [30] that the prenylation step occurs after the formation of the 6a-hydroxypterocarpan glycinol. Glycinol could not be detected in *P. lunatus*, although we have isolated it, in small quantities, earlier from *P. mungo* and *P. coccineus*. The apparent lack of this compound in *P. lunatus* could simply reflect a small pool size.

Unlike *P. vulgaris* [26] and *P. coccineus* [4], *P. lunatus* does not appear to produce the cyclo-derivative of phaseollidin, phaseollin, in response to stress. The presence of enzymes capable of cyclizing γ,γ-dimethylallyl chains at C-3′ in the isoflavone and isoflavanone series, is indicated by the isolation of lunatone, cyclokievitone and cyclokievitone hydrate. In this respect, *P. lunatus* is similar to *P. aureus* [2] and *P. mungo* [3]. *Phaseolus lunatus* also produces the methoxylated isoflavanone isoferreirin (**2**), a feature found so far only in *P. mungo* [3] and *P. coccineus* [4]. Thus taxonomically, the isoflavonoids of *P. lunatus* place it somewhere between *P. vulgaris*/*P. coccineus* and *P. mungo*/*P. aureus*. *Phaseolus lunatus* is known to differ somewhat from *P. vulgaris* and *P. coccineus* in its protein and cross-fertilization characteristics [31, 32]. Whereas some papilionate legumes contain isoflavonoids as constitutive, or pre-infectional compounds, *P. lunatus* is similar to other *Phaseolus* species in tending to produce extractable quantities of these compounds only when stressed. In the present study the kievitone concentration in cuprous chloride-treated seedlings increased up to 5 days after treatment.

EXPERIMENTAL

General. EIMS: direct insertion, 70 eV, inlet temperatures of 170–240°; ^{1}H NMR: 250 or 400 MHz using Fourier Transform techniques; ^{13}C NMR: 400 MHz.

Plant material and extraction of isoflavonoids. Seeds of *P. lunatus*, supplied by Thompson and Morgan Ltd., London were authenticated [33], germinated, treated with aq. $CuCl_2$ and extracted as described previously for other *Phaseolus* species [2, 3]. $CuCl_2$-treated seedlings (4.3 kg) produced 12.4 g EtOAc extract. TLC-bioassay against *Cladosporium cucumerinum* [2] revealed the presence of several fungitoxic substances in the EtOAc extract from $CuCl_2$-treated seedlings. No appreciable fungitoxicity was observed in EtOAc extracts from untreated seedlings. EtOAc extract from $CuCl_2$-treated seedlings was chromatographed over polyamide eluted with a $CHCl_3$–MeOH gradient and yielded 12 fractions which were further purified by TLC over silica gel GF_{254} using the following solvents: hexane–EtOAc–MeOH (6:4:1), solvent A; $CHCl_3$, solvent B; hexane–$CHCl_3$–MeOH (8:12:3), solvent C; hexane–$CHCl_3$–MeOH (12:8:3), solvent D; hexane–$CHCl_3$–MeOH (10:10:3), solvent E, hexane–$CHCl_3$–MeOH (9:11:3), solvent F; $CHCl_3$–*iso*-PrOH (9:1), solvent G. Isoflavonoids were detected using Fast Blue Salt B spray reagent [34] and their yields and distribution in column fractions and TLC R_f values are given in Table 1.

Compounds **1**–**16** were characterized by a comparison of their spectroscopic properties with those of authentic compounds and literature values: **1** [19, 20], **2** [35], **3** [11], **4** [3, 36], **5** [11], **6** [3], **7** [12], **8** [26], **9**, **10** [14], **11** [11], **12** [37], **13** [38, 39], **14** [40], **15** [41], **16** [10, 42, 43].

Numbering of carbons in prenyl chains. The double prime notation has been used throughout for carbons in prenyl chains. Thus C-1″–C-5″ denote carbons in the first prenyl chain:

Table 1. Chromatographic properties and yields of isoflavonoids from *P. lunatus*

Compound	Polyamide column fraction	Solvent systems A	B	C	D	E	F	G	Yield (μg/g fr. wt)
Isoflavones									
Daidzein (7)	3	0.25	—	—	—	0.65	—	0.40	0.48
2′-Hydroxydaidzein (8)	7	0.50	—	—	—	—	—	0.40	0.25
7,8,2′,4′-Tetrahydroxyisoflavone (17)	3	0.25	—	—	—	0.85	—	0.35	0.23
Genistein (9)	4	0.50	—	—	—	—	—	0.55	0.56
2′-Hydroxygenistein (10)	12	0.60	—	—	—	—	0.60	0.56	0.51
5,7,8,2′,4′-Pentahydroxyisoflavone (18)	7	0.35	—	—	—	—	—	0.45	0.12
Luteone (12)	8	0.65	—	—	—	—	—	0.70	0.07
2,3-Dehydrokievitone (11)	10	0.70	—	—	0.39	—	—	0.85	0.08
2,3-Dehydrokievitol (20)	10	0.70	—	—	0.35	—	—	0.92	0.02
Lunatone (21)	2	0.20	—	—	—	—	—	0.50	0.10
Isoflavanones									
Isoferreirin (2)	3	0.35	—	—	0.85	—	—	0.59	0.23
5-Deoxykievitone (3)	7	0.50	—	—	—	—	—	0.55	0.13
5-Deoxykievitol (22)	3	0.23	—	—	—	—	—	0.20	0.19
Kievitone (1)	10	0.60	—	—	0.60	—	—	0.65	8.32
Kievitol (23)	10	0.45	—	—	—	—	—	0.55	2.13
Kievitonehydrate (4)	8	0.25	—	—	—	—	—	0.40	0.97
Cyclokievitone (5)	3	0.45	—	—	—	0.89	—	0.60	0.05
Cyclokievitonehydrate (6)	3	0.25	—	—	—	0.65	—	0.35	0.47
3′(γ,γ-dimethylallyl)-Kievitone (24)	3	0.64	—	—	—	—	—	0.94	0.47
Pterocarpans									
Phaseollidin (13)	2	0.50	—	0.43	—	—	—	0.85	0.04
2-(γ,γ-dimethylallyl)-Phaseollidin (14)	1	0.85	0.45	—	—	—	—	—	0.04
4(γ,γ-dimethylallyl)-Phaseollidin (27)	1	0.85	0.55	—	—	—	—	—	0.06
2-(γ,γ-dimethylallyl)-6a-Hydroxyphaseollidin (28)	3	0.45	—	—	—	—	0.69	0.50	0.11
Coumestans									
Coumestrol (15)	12	0.50	—	—	—	—	0.65	—	0.06
Psoralidin (16)	2	0.43	—	—	0.65	—	—	0.80	0.09

$C^{1''}H_2 \cdot C^{2''}H{=}C^{3''}$ $(Me)_2^{4'',5''}$. Second prenyl chains in a molecule are indicated by C-6″–C-10″.

7,8,2′4′-Tetrahydroxyisoflavone (17). UV λ_{max}^{MeOH} nm: 206, 244 sh, 251, 265 sh, 310 sh; MeOH + NaOMe: 206, 259, 300 sh; MeOH + NaOAc: 254, 300 sh; MeOH + NaOAc + H_3BO_3: 249, 265 sh, 310 sh; MeOH + $AlCl_3$: 206, 241 sh, 255, 268 sh, 296; MeOH + $AlCl_3$ + HCl: 206, 244 sh, 251 sh, 265 sh, 310 sh; ^{1}H NMR (250 MHz, Me_2CO-d_6): δ8.09 (1H, *s*, H-2), 8.06 (1H, *d*, *J* = 8.7 Hz, H-5), 7.43 (1H, *d*, *J* = 8.6 Hz, H-6′), 6.98 (1H, *dd*, *J* = 8.8, 2.5 Hz, H-5′) 6.90 (1H, *d*, *J* = 2.0 Hz, H-3′). 6.87 (1H, *d*, *J* = 8.8 Hz, H-6).

5,7,8,2′,4′-Pentahydroxyisoflavone (18). UV λ_{max}^{MeOH} nm: 206, 265; MeOH + NaOMe: 207, 282; MeOH + NaOAc: 278; MeOH + NaOAc + H_3BO_3: 206, 265; MeOH + $AlCl_3$: 206, 275, 315 sh; MeOH + $AlCl_3$ + HCl: 206, 270; ^{1}H NMR (250 MHz, Me_2CO-d_6): δ 12.65 (1H, *s*, C-5 OH), 8.25 (1H, *s*, H-2), 7.15 (1H, *d*, *J* = 8.1 Hz, H-6′), 6.49 (1H, *d*, *J* = 2.3 Hz, H-3′), 6.44 (1H, *dd*, *J* = 8.0, 2.4 Hz, H-5′), 6.38 (1H, *s*, H-6); NOE: irradiation at 12.65 (C-5 OH) produced enhancement at 6.38 (H-6).

2,3-Dehydrokievitol (20). UV λ_{max}^{MeOH} nm: 266, 290 sh; EIMS *m/z* (rel. int.): 370 (27) $[M]^+$, 354 (35), 353 (100), 352 (93), 339 (31), 338 (68), 337 (87), 336 (32), 335 (41), 319 (31), 311 (45), 300 (35), 299 (72), 287 (24), 286 (27), 219 (64), 218 (24), 204 (35), 203 (88), 190 (32), 177 (40), 165 (69), 161 (63), 153 (31), 136 (24); 135 (47), 134 (84); ^{1}H NMR (250 MHz, Me_2CO-d_6): δ8.25 (1H, *s*, H-2), 7.13 (1H, *d*, *J* = 8.3 Hz, H-6′), 6.49 (1H, *d*, *J* = 2.4 Hz, H-3′), 6.45 (1H, *dd*, *J* = 8.2, 2.4 Hz, H-5′), 6.36 (1H, *s*, H-6), 5.34 (1H, *t*, *J* ~ 7.6 Hz, H-2″), 4.32 (2H, *s*, H-4″), 3.56 (2H, *d* (*br*), *J* ~ 7.6 Hz, H-1″), 1.74 (3H, *s*, H-5″).

Lunatone (21). UV λ_{max}^{MeOH} nm: 206, 266, 350 sh, MeOH + NaOMe: 206, 270; MeOH + NaOAc: 267; MeOH + $AlCl_3$: 206, 274, 340 sh; EIMS *m/z* (rel. int.): 370 (100) $[M]^+$, 352 (22), 337 (31); 324 (12), 312 (27), 311 (62), 287 (15), 221 (6), 219 (6), 218 (6), 203 (21), 177 (21), 176 (15); 150 (44), 149 (50), 137 (44), 135 (47), 134 (21); ^{1}H NMR (250 MHz, Me_2CO-d_6): δ8.16 (1H, *s*, H-2), 711 (1H, *d*, *J* = 8.3 Hz, H-6′), 6.45 (1H, *d*, *J* = 2.2 Hz, H-3′), 6.44 (1H, *dd*, *J* = 8.3, 2.2 Hz, H-5′), 6.24 (1H, *s*, H-6), 4.88 (1H, *dd*, *J* = 8.0, 6.0 Hz, H-2″), 3.30 (2H, *dd*, *J* = 7.9, 6.0 Hz, H-1″), 1.30 (3H, *s*, H-4″), 1.26 (3H, *s*, H-5″); NOE (400 MHz, Me_2CO-d_6): first expt: irradiation at δ1.28 (H-5″) produced enhancement at δ3.32 (2H, H-1″) and 4.89 (1H, H-2″): ratio of enhancements at δ3.32 to 4.89 is 0.68 to 1; second expt: irradiation at δ1.30 (H-4″) produced enhancement at δ3.32 (2H, H-1″) and 4.89 (1H, H-2″): ratio of enhancements at δ3.32 to 4.89 is 0.50 to 1; third expt: irradiation at δ4.89 (1H, H-2″) produced enhancement at δ1.30 (3H, H-4″), 1.28 (3H, H-5″) and 3.32 (2H, H-1″): ratio of enhancements at δ1.30 to 1.28 to 3.32 is 0.47 to 0.44 to 1.

5-Deoxykievitol (22). UV λ_{max}^{MeOH} nm: 206, 286; EIMS *m/z* (rel. int.): 356 (14) $[M]^+$, 340 (21), 338 (29), 322 (22), 321 (11), 305 (15), 284 (9), 270 (39), 253 (40), 221 (15), 205 (58), 203 (100), 187 (15),

167 (23), 163 (15), 161 (29), 153 (15), 150 (22), 147 (14), 137 (50), 136 (51), 135 (33), 134 (31), 107 (31), 105 (22); ^{1}H NMR (250 MHz, Me_2CO-d_6): δ7.66 (1H, *d*, *J* = 8.6 Hz, H-5), 6.98 (1H, *d*, *J* = 8.5 Hz, H-6'), 6.61 (1H, *d*, *J* = 8.6 Hz, H-6), 6.44 (1H, *d*, *J* = 2.5 Hz, H-3'), 6.35 (1H, *dd*, *J* = 8.4, 2.5 Hz, H-5'), 4.70 (1H, *t*, *J* ~ 11.0 Hz, H-2a), 4.70 (1H, *t*, *J* ~ 7.6 Hz, H-2"), 4.61 (1H, *dd*, *J* = 11.1, 5.2 Hz, H-2b), 4.29 (2H, *s*, H-4"), 4.15 (1H, *dd*, *J* = 10.7, 5.3 Hz, H-3), 3.45 (2H, *d* (*br*), *J* = 7.7 Hz, H-1"), 1.75 (3H, *s*, H-5").

Kievitol (**23**). UV λ_{max}^{MeOH} nm: 219, 294, 345 sh; EIMS *m/z* (rel. int.) 372 (5) $[M]^+$, 354 (50), 339 (13), 336 (15), 321 (22), 290 (61), 289 (22), 219 (100), 203 (15), 192 (17), 177 (50), 165 (15), 162 (17), 136 (31), 127 (15), 123 (20), 107 (15); ^{1}H NMR (250 MHz, Me_2CO-d_6): δ6.96 (1H, *d*, *J* = 8.4 Hz, H-6'), 6.44 (1H, *d*, *J* = 2.4 Hz, H-3'), 6.34 (1H, *dd*, *J* = 8.3, 2.4 Hz, H-5'), 6.01 (1H, *s*, H-6), 5.30 (1H, *t*, *J* ~ 7.3 Hz, H-2"), 4.64 (1H, *t J* ~ 10.5 Hz, H-2a), 4.54 (1H, *dd*, *J* = 10.5, 5.4 Hz, H-2b), 4.26 (2H, *s*, H-4"), 4.23 (1H, *dd*, *J* = 10.3, 5.3 Hz, H-3), 3.33 (2H, *d* (*br*), *J* ~ 7.4 Hz, H-1"), 1.73 (3H, *s*, H-5").

3'-(γ,γ-dimethylallyl)-Kievitone (**24**). UV λ_{max}^{MeOH} nm: 255 sh, 295, 350 sh; MeOH + NaOMe: 255 sh, 337; MeOH + NaOAc: 255 sh, 338; MeOH + $AlCl_3$: 317; EIMS *m/z* (rel. int.): 424 (51) $[M]^+$, 405 (22), 390 (9), 350 (13), 335 (8), 325 (5), 323 (5), 313 (7), 295 (12), 294 (13), 293 (16), 292 (14), 221 (77), 205 (23), 204 (22), 177 (33), 165 (100), 149 (35), 135 (19); ^{1}H NMR (250 MHz, Me_2CO-d_6 + $CDCl_3$): δ7.07 (1H, *d*, *J* = 8.5 Hz, H-6'), 6.42 (1H, *d*, *J* = 8.5 Hz, H-5'), 5.99 (1H, *s*, H-6), 5.27 (2H, *m*, H-2", H-7"), 4.80 (1H, *t*, *J* ~ 11.5 Hz, H-2a), 4.68 (1H, *dd*, *J* = 11.6, 4.7 Hz, H-2b), 4.00 (1H, *dd*, *J* = 11.6, 4.9 Hz, H-3), 3.40 (2H, *d* (*br*), *J* = 7.0 Hz, H-1"), 3.23 (2H, *d* (*br*), *J* = 7.1 Hz, H-6"), 1.77 (6H, *s*, H-4", H-9"), 1.67 (6H, *s*, H-5", H-10"); ^{13}C NMR (400 MHz, Me_2CO-d_6): δ129.90 (C-6'), 122.29 (C-2", C-7"), 121.33 (C-1'), 106.80 (C-5', C-8), 105.60 (C-3'), 100.50 (C-4a), 95.20 (C-6), 69.19 (C-2), 44.58 (C-3), 24.16 (C-4", C-9"), 21.63 (C-1"), 20.41 (C-6"), 16.20 (C-5", C-10").

4-(γ,γ-dimethylallyl)-Phaseollidin (**27**). UV λ_{max}^{MeOH} nm: 282 sh, 286; MeOH + NaOMe: 282 sh, 286, 342; MeOH + NaOAc: 282 sh, 286, 342; EIMS *m/z* (rel. int.): 392 (100) $[M]^+$, 377 (3), 375 (4), 349 (3), 336 (27), 320 (13), 292 (15), 280 (54), 202 (7), 189 (15), 167 (4), 161 (15), 152 (3), 147 (13), 135 (21); ^{1}H NMR (400 MHz, $CDCl_3$): δ7.30 (1H, *d*, *J* = 8.4 Hz, H-1), 6.98 (1H, *d*, *J* = 8.5 Hz, H-7), 6.58 (1H, *d*, *J* = 8.4 Hz, H-8), 6.38 (1H, *d*, *J* = 8.4 Hz, H-2), 5.48 (1H, *d*, *J* = 7.0 Hz, H-11a), 5.35 (1H, *s*, C-9 OH), 5.29 (2H, *m*, H-2", C-3 OH), 5.22 (1H, *m*, H-7"), 4.28 (1H, *dd*, *J* = 11.6, 5.6 Hz, $H\text{-}6_{ax}$), 3.60 (1H, *t* (*br*), *J* ~ 11.3 Hz, $H\text{-}6_{eq}$), 3.50 (1H, *m*, H-6a), 3.45 (4H, *m*, H-1", H-6"), 1.81 (6H, *s*, H-4", H-9"), 1.74 (6H, *s*, H-5", H-10"); D_2O exchange at δ5.29, 5.35.

2-(γ,γ-dimethylallyl)-6a-Hydroxy-phaseollidin (**28**). UV λ_{max}^{MeOH} nm: 284 sh, 289; ^{1}H NMR (250 MHz, $CDCl_3$): δ7.22 (1H, *s*, H-1), 7.08 (1H, *d*, *J* = 8.1 Hz, H-7), 6.44 (1H, *d*, *J* = 8.2 Hz, H-8), 6.40 (1H, *s*, H-4), 5.30 (2H, *m*, H-2", H-7"), 5.24 (1H, *s*, H-11a), 4.17 (1H, *d*, *J* = 11.6 Hz, $H\text{-}6_{ax}$), 3.94 (1H, *d*, *J* = 11.6 Hz, $H\text{-}6_{eq}$), 3.34 (4H, *d* (*br*), *J* ~ 7.0 Hz, H-1", H-6"), 1.76 (6H, *s*, H-4", H-9"), 1.74 (6H, *s*, H-5", H-10").

2,10-Di-(γ,γ-dimethylallyl)-3,9-dihydroxy-pterocarpene (**31**). UV λ_{max}^{MeOH} nm: 210, 245 sh, 252 sh, 330 sh, 342, 356 sh: MeOH + NaOMe: 207, 245 sh, 252 sh, 347, 356 sh; EIMS *m/z* (rel. int.): 390 (100) $[M]^+$, 389 (12), 335 (26), 334 (29), 291 (6), 279 (40), 251 (5), 165 (6), 160 (5), 153 (4), 152 (6), 140 (5).

Acknowledgements—We thank Dr. A. I. Gray, University of Strathclyde, for helpful discussions on the NMR of cyclokievitone hydrate, Dr. G. Hawkes, Queen Mary College, and Ms. J. Elliott, King's College, University of London for NMR determinations and Mr. D. Carter and Mr. K. Welham for MS. We are grateful to Mrs. A. Cavanagh for drawings and Mrs. J. Hallsworth for typing the manuscript. We acknowledge financial support from the Federal Government of Nigeria (to S.A.A.) and the Pharmaceutical Society of Great Britain (to I.R.P.).

REFERENCES

1. Ingham, J. L. (1982) in *Phytoalexins* (Bailey, J. A. and Mansfield J. W., eds) p. 21. Blackie, London.
2. O'Neill, M. J., Adesanya, S. A. and Roberts, M. F. (1983) *Z. Naturforsch.* **38c**, 693.
3. Adesanya, S. A., O'Neill, M. J. and Roberts, M. F. (1984) *Z. Naturforsch.* **39c**, 888.
4. Adesanya, S. A., O'Neill, M. J. and Roberts, M. F. (1985) *Phytochemistry* **24**, 2699.
5. Gordon, M. A., Lapa, E. W., Fitter, M. S. and Lindsay, M. (1980) *Antimicrob. Agents Chemother.* **17**, 120.
6. Haenen, J. M. (1985) *Pharmacy International*, August, 194.
7. Kay, D. E. (ed.) (1979) *Food Legumes, Crop and Product Digest*, No. 3. Tropical Products Institute, London.
8. Duke, J. A. (1983) *Handbook of Legumes of World Economic Importance*, p. 189. Plenum Press, London.
9. Partridge, J. E. (1973) *Fungus Disease Defense Responses in Legumes*. Ph.D. dissertation, University of California, Riverside, U.S.A.
10. Rich, J. R., Keen, N. T. and Thomason, I. J. (1977) *Physiol. Plant Pathol.* **10**, 105.
11. Woodward, M. D. (1979) *Phytochemistry* **18**, 2007.
12. Keen, N. T. and Kennedy, B. W. (1974) *Physiol. Plant Pathol.* **4**, 173.
13. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
14. Biggs, D. R. (1975) *Aust. J. Chem.* **28**, 1389.
15. Markham, K. R., Mabry, T. J. and Swift, T. W. (1968) *Phytochemistry* **7**, 803.
16. Nakagawa, M., Nakanishi, K., Darko, L. L. and Vick, J. A. (1982) *Tetrahedron Letters* 3855.
17. Bell, A. A. and Saunders, J. K. (1970) *Can. J. Chem.* **48**, 114.
18. Pachler, K. G. R. and Underwood, W. G. E. (1967) *Tetrahedron* **23**, 1817.
19. Smith, D. A., Van Etten, H. D., Serum, J. W., Jones, T. M., Bateman, D. F., Williams, T. H. and Coffen, D. L. (1973) *Physiol. Plant Pathol.* **3**, 293.
20. Burden, R. S., Bailey, J. A. and Dawson, G. W. (1972) *Tetrahedron Letters* 4175.
21. Komatsu, M., Yokoe, I. and Shirataki, Y. (1978) *Chem. Pharm. Bull.* **26**, 3865.
22. Delle Monache, G., Delle Monache, F., Marini-Bettolo, G. B., De Albuquerque, M., De Mello, J. and De Lima, O. G. (1977) *Gazz. Chim. Ital.* **107**, 189.
23. Dewick, P. M., Steele, M. J., Dixon, R. A. and Whitehead, I. M. (1982) *Z. Naturforsch.* **37c**, 363.
24. Ingham, J. L. and Markham, K. R. (1980) *Phytochemistry* **19**, 1203.
25. Lyne, R. W. and Mulheirn, L. J. (1978) *Tetrahedron Letters* 3127.
26. Woodward, M. D. (1980) *Phytochemistry* **19**, 921.
27. Woodward, M. D. (1979) *Phytochemistry* **18**, 363.
28. Dewick, P. M. and Steele, M. J. (1982) *Phytochemistry* **21**, 1599.
29. Dewick, P. M. (1978) *Phytochemistry* **17**, 249.
30. Banks, S. W. and Dewick, P. M. (1983) *Phytochemistry* **22**, 2729.
31. Kloz, J. (1962) *Biol. Plant.* **4**, 85.
32. Kloz, J. and Klozova, E. (1974) *Biol. Plant.* **16**, 290.
33. Marechal, R., Mascherpa, J. and Stainer, F. (1978) *Boisseira* **28**, 1.
34. Stahl, E. (1969) *Thin Layer Chromatography*, 2nd edn. Springer, Berlin.
35. Keen, N. T. and Ingham, J. L. (1980) *Z. Naturforsch.* **35c**, 923.

36. Kuhn, P. J., Smith, D. A. and Ewing, D. F. (1977) *Phytochemistry* **16**, 296.
37. Fukui, H., Egawa, H., Koshimizu, K. and Mitsui, T. (1973) *Agric. Biol. Chem.* **37**, 417.
38. Perrin, D. R., Whittle, C. P. and Batterham, T. J. (1972) *Tetrahedron Letters* 1673.
39. Perrin, D. R. and Cruickshank, I. A. M. (1974) *Aust. J. Chem.* **27**, 1607.
40. Kamat, V. S., Chuo, F. Y., Kubo, I. and Nakanishi, K. (1981) *Heterocycles* **15**, 1163.
41. Bickoff, E. M., Spencer, R. R., Witt, S. C. and Knuckles, B. E. (1969) *U.S.A. Dept. Agric. Tech. Bull.* **1408**, 1.
42. Khastgir, H. N., Duttagupta, P. C. and Sengupta, P. (1961) *Tetrahedron* **14**, 275.
43. Abdel-Hay, F. M., Abu-Mustafa, E. A. and Fayez, M. B. E. (1967) *Rec. Trav. Chim. Pays-Bas* **86**, 920.