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ISOFLAVONES FROM AN INSECT-RESISTANT VARIETY OF SOYBEAN AND THE MOLECULAR STRUCTURE OF AFRORMOSIN

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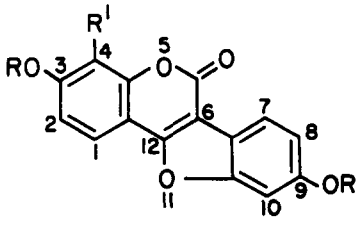
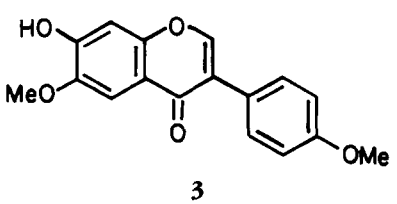
In our search for the chemical basis of resistance of the soybean variety PI227687 *Glycine max* Mer. (Leguminosae) to the soybean looper *Pseudoplusia includens*, we have isolated and chemically characterized three isoflavonoids from two active fractions of a soybean foliage MeOH extract. *P. includens* mortality caused by fractions 3 and 4 in an artificial diet were 71 and 98%, respectively. Current experiments involve the synthesis of these isoflavones in order to obtain biological data on the pure compounds.

Coumestrol (**1**), a common isoflavonoid first isolated from ladino clover (1), was isolated as a solid from fraction 3. Characterization of coumestrol involved ^{1}H-nmr (200 MHz) and ms comparison of its acetate with authentic material. Phaseol (**2**) was isolated as a solid from fraction 3. It was characterized by 200 MHz ^{1}H-nmr and ms analyses of its acetate derivative. The ^{1}H-nmr spectrum in $CDCl_3$exhibited characteristic signals that were in accord with reported data (2). This is the first report of the presence of phaseol (**2**) in soybean.



1 R=R^1=H
1a R=Ac, R^1=H
2 R=H, R^1=prenyl
2a R=Ac, R^1=prenyl



3

The common isoflavone afrormosin (**3**) (3) was isolated from fraction 4. Characterization involved ^{1}H-nmr and ms analyses, and the molecular structure was determined by single crystal X-ray analysis.

The structure of afrormosin is illustrated in Figure 1. The isoflavone fused ring system is planar with all ten atoms lying an average of 0.004Å from a common plane and the carbonyl oxygen atom lying 0.016(1)Å out of this plane. The six atoms of the phenyl substituent lie an average of 0.010Å from a common plane, and this plane forms a dihedral angle of 53.9° with that of the fused ring system. Bond distances are normal with C-C lengths in the C(5) through C(10) ring ranging 1.364(4)-1.415 (3)Å and averaging 1.389Å, and corresponding distances within the phenyl substituent ranging 1.372 (4)-1.394(4)Å and averaging 1.382Å. The C(2)=C(3) double bond has a length of 1.334(4)Å, the carbonyl C(4)=O(11) 1.242(3)Å, and bonds to O(1) are asymmetric, O(1)-C(2) 1.349(3)Å, O(1)-C(9) 1.381(3)Å. Molecules are linked in the solid by linear hydrogen bonds O(13)-H. . . .O(11), with length 2.695(3)Å, H. . . .O distance 1.73(4)Å, and angle at H 174(3)°.

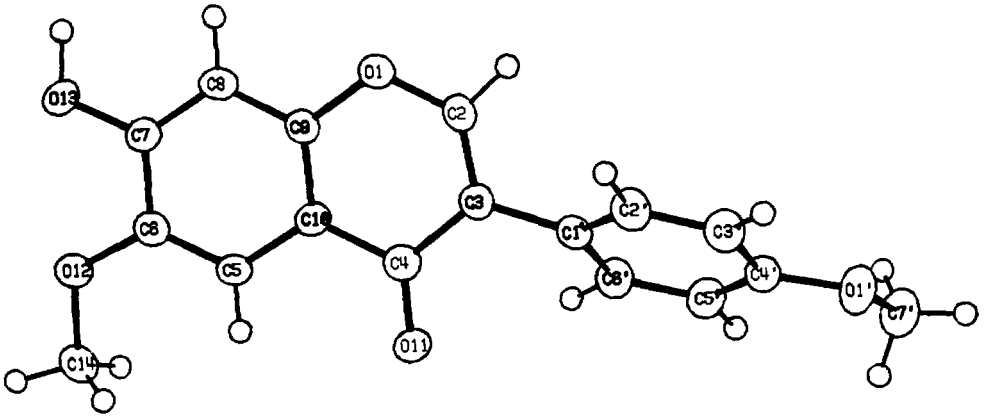


FIGURE 1. The crystal structure of afformosin

EXPERIMENTAL

PLANT MATERIAL AND EXTRACTION.—Seeds of soybean, genotype PI227687, were obtained from Dr. Curtis Williams, Jacob Hartz Seed Co., Stuttgart, AR, and were field-planted in June 1983. When plants reached the V7 stage (4), the fourth through eighth trifoliates down from plant apices were collected, air dried, ground to a fine powder, and extracted in a Soxhlet apparatus in a series with petroleum ether, CH_2Cl_2, and MeOH for 48 h each. The residue was then stirred with H_2O for another 48 h. The MeOH extract (10 g) was fractionated by flash chromatography on 250 g of Si gel. The column was initially developed with $CHCl_3$ until no material was eluted. This fraction was called 4 and yielded 0.43 g. A gradient solvent of 500-ml volume with 10% increments of Me_2CO was used later; 250-ml fractions were collected and monitored by tlc on analytical Si gel plates using $CHCl_3$-Me_2CO (10:1) as eluent and recombined in fractions 3 (0.710 g) and 2 (0.7 g). Fraction 1 (8.14 g) was eluted with pure MeOH.

In order to eliminate chlorophyll from fractions 3 and 4, flash chromatography on a C-18 reverse phase Si gel column was performed using MeOH-H_2O (4:1) as eluent. Of fraction 3, 100 mg was applied to a 1-mm reverse phase tlc plate and developed with MeOH-H_2O (4:1). Coumestrol (15 mg) and phaseol (22 mg) were isolated as solids with Rf's of 0.4 and 0.27, respectively. Of fraction 4, 100 mg was applied to a 1-mm normal phase tlc Si gel plate and developed with hexane-Me_2CO (2:1) followed by elution with $CHCl_3$-MeOH (25:1). Afformosin (15 mg) was isolated as crystals with an overall Rf of 0.62.

COUMESTROL ACETATE (**1a**).—$C_{19}H_{12}O_7$; ^{1}H nmr (200 MHz, $CDCl_3$) H-1 (δ 8.02 d, $J_{1,2}$=8.5 Hz), H-2 (δ7.2 dd, $J_{1,2}$=8.5; $J_{2,4}$=2.3 Hz), H-4 (δ 7.3 d, $J_{4,2}$=2.3 Hz), H-7 (δ 8.11 d, $J_{7,8}$=8.6 Hz), H-8 (δ 7.22 dd, $J_{8,7}$=8.6; $J_{8,10}$=2.4 Hz), H-10 (δ 7.49 d, $J_{10,8}$=2.40 Hz), CH_3 (δ 2.43, s); ms 70 eV *m/z* (rel. int.) 352 (6.2, $C_{19}H_{12}O_7$, M^+), 310 (11.2, M^+-C_2H_2O), 268 (100, M^+-$2C_2H_2O$).

PHASEOL ACETATE (**2b**).—$C_{24}H_{20}O_7$; ^{1}H nmr (200 MHz, $CDCl_3$), H-1 (δ 7.85 d, $J_{1,2}$=8.5 Hz), H-2 (δ 7.6 d, $J_{2,1}$=8.5 Hz), H-7 (δ 8.12 d, $J_{7,8}$=7,8=8.7 Hz), H-8 (δ 7.21 dd, $J_{8,7}$=8.7; $J_{8,10}$=2.2 Hz), H-10 (δ 7.48, d, $J_{10,8}$=2.2 Hz), H-1′ (δ 3.59 d, $J_{1',2'}$=7.0 Hz), H-2′ (δ 5.19 brt, $J_{2',1'}$=7.0 Hz), H-3′a (δ 1.84 s), H-3′b (δ 1.70, s), CH_3 (δ 2.43, s); ms 70 eV *m/z* (rel. int.) 420 (6.7, M^+), 378 (8.5, M^+-C_2H_2O), 336 (46, M^+-$2C_2H_2O$), 281 (21, M^+-$2C_2H_2O$-C_4H_7), 280 (60, M^+-$2C_2H_2O$-C_4H_8).

AFRORMOSIN (**3**).—$C_{17}H_{14}O_5$; ^{1}H nmr (200 MHz, $CDCl_3$), H-2 (δ 8.35 s), H-5 (δ 7.61 s), H-8 (δ 6.93 s), H-2′, H-6′ (δ 7.66 d, $J_{2',3'}$=$J_{6',5'}$=8.5 Hz), H-3′, H-5′ (δ 6.97 d, $J_{3',2'}$=$J_{6',5'}$=8.5 Hz); ms *m/z* (rel. int.) 298 (100, M^+), 283 (11, M^+-CH_3), 267 (1.5, M^+-CH_3O), 166 (44, M^+-C_9H_8O).

X-RAY DATA OF AFRORMOSIN (**3**)[1].—A crystal of dimensions 0.10×0.16×0.32 was used for data collection on an Enraf-Nonius CAD4 diffractometer equipped with MoKα radiation and a graphite monochromator. Crystal Data: $C_{17}H_{14}O_5$, MW=298.3, orthorhombic space group $Pca2_1$, a=27.470(4), b=6.505(1), c=7.533(3)Å, V=1346.1(9)Å^3, Z=4, D_c =1.472 gcm^{-3}, λ=0.71073Å, μ (MoKα)

[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

TABLE 1. Coordinates and Equivalent Isotropic Thermal Parameters for Afrormosin

Atom	x	y	z	Beq	Atom	x	y	z	Beq
O(1)	0.66929(6)	0.3741(3)	0.2303(3)	3.08(4)	O(12)	0.86059(6)	0.1555(3)	0.1470(3)	3.43(4)
C(2)	0.63328(9)	0.2485(4)	0.1750(5)	2.93(6)	O(13)	0.83805(7)	0.5000(3)	0.3020(3)	3.32(4)
C(3)	0.63872(9)	0.0653(4)	0.0981(4)	2.53(6)	C(14)	0.87490(9)	−0.0411(5)	0.0797(5)	3.50(7)
C(4)	0.68746(9)	−0.0158(4)	0.0686(4)	2.61(6)	C(1′)	0.59361(9)	−0.0482(4)	0.0485(4)	2.56(6)
C(5)	0.77615(9)	0.0644(4)	0.1070(4)	2.47(6)	C(2′)	0.55734(9)	0.0457(4)	−0.0524(5)	3.02(6)
C(6)	0.81218(9)	0.1934(4)	0.1627(4)	2.55(6)	C(3′)	0.51397(9)	−0.0516(4)	−0.0856(5)	3.31(7)
C(7)	0.80056(9)	0.3831(4)	0.2437(4)	2.43(6)	C(4′)	0.50515(9)	−0.2449(4)	−0.0173(4)	2.74(6)
C(8)	0.75269(12)	0.4405(4)	0.2614(4)	2.55(6)	C(5′)	0.54084(9)	−0.3434(4)	0.0787(5)	3.31(7)
C(9)	0.71660(9)	0.3086(4)	0.2054(4)	2.32(6)	C(6′)	0.58483(9)	−0.2458(4)	0.1091(5)	3.18(7)
C(10)	0.72660(9)	0.1190(4)	0.1277(4)	2.36(6)	O(1′)	0.46016(7)	−0.3288(3)	−0.0537(3)	3.78(5)
O(11)	0.69577(6)	−0.1859(3)	0	3.23(4)	C(7′)	0.44546(11)	−0.5044(5)	0.0468(5)	4.14(8)

$=1.02$ cm^{-1}, $T=20°$. Data were collected by ω-2θ scans of variable speed ranging 0.20-5.0 degrees min^{-1}. One octant of data having $1°<\theta<27°$ was measured, yielding 1582 unique reflections of which 1458 and $I>O$ and were used in the refinement. Data reduction included corrections for background, Lorentz, and polarization effects; no absorption correction was necessary.

The structure was solved by direct methods (MULTAN 78) (5) and was refined by full matrix least squares with weights $w=\sigma^{-2}(Fo)$. C and O atoms were treated anisotropically, while H atoms were located from difference maps and refined isotropically. Convergence was achieved with $R=0.058$, $R_w=0.038$, extinction coefficient $5.2(7)\times10^{-7}$, and maximum residual density 0.26e $Å^{-3}$. Coordinates are given in Table 1.

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