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# NEW STILBENE PHYTOALEXINS FROM AMERICAN CULTIVARS OF *ARACHIS HYPOGAEA*

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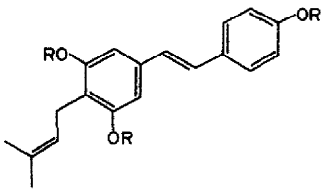
**Key Word Index**—*Arachis hypogaea*; Leguminosae; stilbene; phytoalexins; antifungal compounds.

**Abstract**—Two phytoalexins from American varieties of *Arachis* have been characterized as the *cis*- and *trans*-isomers of 3,5,4′-trihydroxy-4-isopentenylstilbene.

Keen [1] previously reported that two antifungal compounds were present in extracts from germinating seeds or from stems of American peanut (*Arachis hypogaea* L., Virginia Jumbo and Spanish, obtained from Burpee Seed Co., Riverside, CA, U.S.A.) plants challenged by microorganisms. Simultaneously, Ingham isolated two related but distinct compounds from the fungus-infected hypocotyls of an African-grown cultivar of *A. hypogaea*. These have been identified as *cis*- and *trans*-3,5,4′-trihydroxystilbene (resveratrol) [2]. Since the compounds from American or African peanuts were not detected in extracts from non-inoculated plant tissues, they appeared to be phytoalexins, post-infectionally formed compounds involved in the resistance of plants to attack by microorganisms [3,4]. We report evidence here indicating that the phytoalexins from American peanuts are the *cis*- and *trans*-isomers of 3,5,4′-trihydroxy-4-isopentenylstilbene (**1a**), compounds previously not found in nature.

The two peanut compounds were blue fluorescing on TLC plates under 254 nm UV light and quickly decomposed in daylight to form yellow products that were not antifungal. For purification, the compounds were extracted from germinating peanut seeds [1] by grinding in 95% EtOH at room temp. The filtered solutions were concentrated and extracted with EtOAc. The EtOAc fraction was concentrated and chromatographed on Merck $GF_{254}$ Si gel plates (0.375 or 0.5 mm) with hexane–EtOAc–MeOH (60:40:1). The two blue fluorescent bands ($R_f$ 0.47 and 0.28) were eluted with $Me_2CO$, and the concentrated elutes rechromatographed on preparative plates with $CHCl_3$–$Me_2CO$–conc $NH_4OH$ (50:50:1, $R_f$ 0.47 and 0.28, respectively). Elution with $Me_2CO$ gave the two compounds as viscous oils which could not be crystallized and which rapidly turned yellow in air; they were therefore stored in solution.




**1a** R=H
**1b** R=Me

The two compounds gave similar mass spectra ($M^+ = 296$ for both compounds and high resolution gave the empirical formulae as $C_{19}H_{20}O_3$); other prominent fragments at *m/e* 281, 279, 241 (base peak), 165, 137, and 121); the base peak at $M^+ - 55$ was suggestive of an *o*-hydroxy isopentenyl grouping; the lower compound gave $\lambda_{max}$ (EtOH) 302, 216 nm and NaOH produced an absorbance shift to 350 nm; the upper compound gave $\lambda_{max}$ (EtOH) 327, 225 nm with a shift of the high band to 355 nm with NaOH added); both compounds formed triacetates ($M^+ = 422$, $C_{25}H_{26}O_6$). IR spectra of both compounds were nearly identical and suggested aromaticity and the presence of hydroxyl groups. The NMR spectra of the two compounds were very similar and disclosed several aromatic protons and signals typical of isopentenyl groups. Since there appeared to be no heterocyclic oxygen atoms, it was considered that the two compounds might be trihydroxylated, isopentenylstilbenes. If true, a 3,5,4′-hydroxylation pattern was most biogenetically feasible with the isopentenyl residue located at carbon 4; the two peanut compounds were therefore methylated ($Me_2SO_4$, $K_2CO_3$, $Me_2CO$) and the resulting products isolated by TLC and compared with a sample of 3,5,4′-trimethoxy-4-isopentenylstilbene (**1b**), recently isolated from *Derris floribunda* by Braz *et al.* [5]. The upper methylated peanut compound gave identical UV and mass spectra to the *Derris* stilbene and co-chromatographed with it in several Si gel systems. Braz *et al.* [5] formulated their stilbene as the *trans*-isomer (**1b**), and our data support this conclusion. Thus the upper peanut compound is *trans*- 3,5,4′-trihydroxy-4-isopentenylstilbene (**1a**). The other peanut compound is considered to be the corresponding *cis*-isomer on the basis of its consistently lower $R_f$ in TLC systems [6], its lower UV absorption band [6], and the similarity of other spectral data to the *trans*-compound.

The stilbene isomers appeared to have similar antifungal activity against *Cladosporium cucumerinum* in the TLC bioassay [1] (minimum activity = 1–5 μg spotted). Although these two stilbenes have not previously been reported as phytoalexins, it is well known that several related and naturally-occurring hydroxylated stilbenes have marked antifungal properties [7–10]. In agreement with other findings [11], complete acetylation or methylation of the peanut stilbenes destroyed their antifungal activity in the TLC bioassay. Although stilbenes have

been considered to be phytoalexins in the heartwood of several coniferous trees [12], this paper and that of Ingham [2] are the first to report such compounds as phytoalexins in herbaceous plants.

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# PYRANO FLAVANONE FROM *MILLETIA OVALIFOLIA* SEEDS

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**Key Word Index**—*Milletia ovalifolia*; Leguminosae; pongapin; pongamol; new pyranoflavanone.

*Plant. Milletia ovalifolia* (Leguminosae). *Past work.* On leaves and bark [1], on seeds [2], on related species [3,4]. *Present work.* On seeds obtained from L. R. Brothers (Saharanpur), India. In continuation of our earlier work [2] further examination of the seeds led to the isolation of pongapin [5], pongamol [6] and a new pyranoflavanone designated as ovalichromene. It crystallized from $C_6H_6$–petrol as white needles mp 162–163°, $[\alpha]_D^{20}$ −90° and had a formula $C_{21}H_{20}O_4$ ($M^+$ 336). It gave a Mg/HCl colour reaction, $\lambda_{max}^{MeOH}$ nm (log ε), 255 (4.19), 315 (3.64), 345 (3.56), $\nu_{max}^{KBr}$ 1620, 1450, 1320, 1275, 1150, 755, 730 $cm^{-1}$. PMR (δ values solvent $CDCl_3$) showed a single OMe group at 4.0 (*s*, 3 H); two sharp aromatic proton peaks at 7.5 (*s*, 1 H) (H-5) and 7.65 (*s*, 5 H) ($C_6H_5$); two doublets at 6.85 (1 H, $J_{3',4'}$ 10 Hz) and 5.75 (1 H, $J_{3',4'}$ 10 Hz) due to $H_{4'}$ and $H_{3'}$; one aliphatic proton at 5.6 (*m*, H-2); two protons at 3.0 (*m*, H-3 proton) and a sharp singlet at 1.55 (6 H, –$C(Me)_2$–O–). These data indicated a close relationship to the flavanone obtained by cyclization of flemichapparin-A [7] and led to the structure of ovalichromene as 6-methoxy-7,8-(2″,2″-dimethylpyrano (5″,6″)-flavanone.

A synthetic sample was prepared from 2,4-dihydroxy-5-methoxy acetophenone by reaction with 3-methyl-3-chloro-but-1-yne in dioxan–$K_2CO_3$ when 6-acetyl-5-hydroxy-8-methoxy-2,2-dimethyl chromene was obtained as pale yellow needles mp 82–83°; $C_{14}H_{16}O_4$; $\lambda_{max}^{MeOH}$ nm (log ε) 255 (4.31), 320 (3.81), 340 (3.74); $\nu_{max}^{KBr}$ 3450 (*b*), 1620, 1445, 1375, 1280, 1140, 980, 890 $cm^{-1}$. PMR(δ values, $CCl_4$) 7.3 (*s*, 1 H, H-6), two doublets at 7.05 (1 H, *J* 10 Hz) and 5.8 (1 H, *J* 10 Hz) due to two vinylic protons; three sharp singlets at 4.0 (3 H, –OMe), 2.6 (3 H, –COMe) and at 1.6 (6 H, –$C(Me)_2$–O–); chelated –OH appears at (*s*, 12.55) (+$D_2O$ exchangeable). Treatment of the above chromenoketone with benzaldehyde under basic conditions yielded the chalcone and the corresponding flavanone, ovalichromene, which were separated by TLC on Si gel. The chalcone was obtained as orange-red needles mp 106–108°, $C_{21}H_{20}O_4$, $\lambda_{max}^{MeOH}$ nm (log ε), 300 (4.02), 355 (3.89); $\nu_{max}^{KBr}$ 3550 (*br*, 1640, 1570, 1470, 1280, 1150, 960, 885, 760 $cm^{-1}$ PMR (δ $CCl_4$) showed one –OMe at 3.9 (*s*, 3 H) and one chelated –OH at 12.8 (*s*, 1 H) (+$D_2O$ exchangeable), one sharp singlet at 7.55 (1 H, H-6′), multiplet from 7.7–8.15 (7 H) due to –$C_6H_5$ and α, β-protons and one sharp singlet at 1.6 (6 H–$C(Me)_2$–O–). The IR spectrum (in $CHCl_3$) of the synthetic sample was identical with that of the natural product.

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