development of vegetable protein mixtures for supplementing human diets. These mixtures usually consist of combinations of a high protein source and a cereal grain, as is the case with Vegetable Mixture 9. They are to be used to supplement rural diets rich in protein-poor cereal grains. Therefore, the vegetable mixture should consist of the optimum protein combination of the cereal protein and the protein-rich component to give the highest nutritive value that can be obtained. Otherwise, the effect on the rural cereal diet will not be as efficient as expected.

Although the supplementary effect of Vegetable Mixture 9 was not as high as expected, it cannot be concluded that it would not be of value when diets, such as the ones used in this study, are consumed by children. The rat is a faster growing organism than the human and consequently has a higher lysine requirement.

All supplements (except in the Santa Catarina Barahona diet) did not reduce food intake, a further indication of the improvement in quality of the supplemented diets. A disadvantage of vegetable protein mixtures is their bulk and low digestibility.

The supplements, besides improving weights gain and PER, also improved some of the constituents of the tissues analyzed and increased the weight of some of the organs studied. The bone composition data, however, suggest that calcium is not as effectively utilized in these mixtures as the calcium from skim milk; this could be due, to some extent, to the phytin content of the vegetable products, and could explain, to a certain degree, the lower PER values obtained with the mixture as compared to those obtained with skim milk.

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NEW ALFALFA COMPOUND IDENTIFIED

Isolation of 4′-O-Methylcoumestrol from Alfalfa

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The coumestan 4′-O-methylcoumestrol was tentatively identified by thin-layer chromatography (TLC) and comparison of its benzyloxybenzofuran derivative with authentic 2-(2′-methoxy-4′-benzyloxyphenyl)-6-methoxybenzofuran. Comparison of the isolated product with authentic 4′-O-methylcoumestrol confirmed the assigned structure.

In a recent paper (*2*), the isolation of 13 phenolic compounds from alfalfa was reported. Two of these (compounds I and II) were obtained as an inseparable mixture. That the two compounds were closely related coumestans was suggested by the similar ultraviolet spectrum of the mixture to that of coumestrol (*1*). Chromatography established that neither compound was coumestrol (*2*). Formation of two new compounds upon acetylation or methylation of the mixture suggested the presence of hydroxyl groups on both compounds. Compound I was subsequently identified through degradative and proton magnetic resonance (PMR) studies as 7-hydroxy-11,12-methylenedioxycoumestan and assigned the trivial name medicagol (*4*). TLC comparison of the mixture of medicagol and compound II with 7- and 4′-*O*-methylcoumestrol suggested that compound II was 4′-*O*-methylcoumestrol (Table I).

The definite location of the hydroxyl group on compound II was established by the preparation of the benzyloxybenzofuran derivatives of the mixture of medicagol and compound II. These were prepared by the systematic degradation of their benzyloxy derivatives through methylative ring opening, hydrolysis, and decarboxylation to their benzyloxybenzofuran derivatives. The benzofurans were then separated by fractional crystallization. Elemental analyses of the benzyloxybenzofuran derivative of compound II substantiated that the compound from which it was derived was a monomethoxy derivative of coumestrol. Comparison with authentic 2-(2′-methoxy-4′-benzyloxyphenyl)-6-methoxybenzofuran confirmed its identity.

Natural 4′-*O*-methylcoumestrol was obtained by treatment of the mixture of compound II and medicagol with 86% sulfuric acid. Under the conditions employed, medicagol was completely converted to 7,11,12-trihydroxycoumestan, while 4′-*O*-methylcoumestrol re-

Table I. R_f Values[a] of 7 and 4′-O-Methylcoumestrol Compared with Compound II

Solvent System	Average R_f Values: 7-O-Methyl-coumestrol	4′-O-Methyl-coumestrol	Parent phenols of compounds I–II	7-O-Methyl-coumestrol acetate	4′-O-Methyl-coumestrol acetate	Compound II acetate
Chloroform	0.08	0.05	0.05	0.31	0.39	0.39
Acetone–Skellysolve B (1 to 2)	0.46	0.42	0.42	0.54	0.55	0.55
Benzene–anhydrous ether (4 to 1)	0.39	0.20	0.19	0.63	0.67	0.67
Anhydrous ether–Skellysolve B (4 to 1)	0.63	0.51	0.51	0.73	0.77	0.76
Ethyl acetate–Skellysolve B (1 to 1)	0.72	0.52	0.51	0.80	0.81	0.82

[a] TLC on silica gel G.

mained unchanged. Separation of the unchanged 4′-*O*-methylcoumestrol from the trihydroxycoumestan was readily accomplished by countercurrent distribution (CCD). Final confirmation of the structure of the natural product was therefore achieved by comparison with an authentic sample of 4′-*O*-methylcoumestrol. Although this compound and 7-*O*-methylcoumestrol were both synthesized from coumestrol by Jurd at this laboratory several years ago (*3*), to the authors' knowledge, this is the first report of 4′-*O*-methylcoumestrol in nature.

Experimental

Preparation of the Benzofuran Derivatives. The following reactions were carried out under an inert atmosphere and were followed by TLC on silica gel G using a mixture of anhydrous ether and Skellysolve B (7 to 3) as the developer.

ACETYLATION AND METHYLATION. Acetylation and methylation of the natural mixture of medicagol and compound II were described earlier (*2, 4*).

BENZYLATION. A mixture of the acetates of medicagol and 4′-*O*-methylcoumestrol (compounds I and II) (*2*) (1.5 grams), potassium iodide (2.0 grams), anhydrous potassium carbonate (3.0 grams), and dry acetone (800 ml.) was brought to reflux and benzyl chloride (15 ml.) was added dropwise. The mixture was refluxed for $2^1/_2$ hours, then filtered, concentrated to a small volume, and diluted with Skellysolve B. The white solids (1.5 grams) were collected.

METHYLATIVE RING OPENING. The above benzyloxy preparation (1.5 grams), potassium carbonate (2.0 grams), and dry acetone (400 ml.) were brought to reflux, and dimethyl sulfate (3.0 ml.) was slowly added. Ten per cent potassium hydroxide in methanol was added dropwise until the alkali no longer turned the solution yellow. The mixture was refluxed an additional 30 minutes, filtered, concentrated to a small volume, and diluted with ether. The solids (1.5 grams) were collected for use in the next reaction.

HYDROLYSIS TO MIXED ACIDS. The above ester preparation (1.5 grams) was hydrolyzed by refluxing with 10% potassium hydroxide in methanol (300 ml.) for one hour. The mixture was diluted with ice water (1 liter) and acidified, and the white solids (1.4 grams, were collected, washed with water, and dried.

DECARBOXYLATION TO BENZYLOXYBENZOFURANS. The above mixture of acids was decarboxylated by heating at 220–25° C. for 2 hours. The dark residue was dissolved in ether (400 ml.), extracted with 10% sodium carbonate, washed with water, dried (sodium sulfate), and taken to dryness. The solids (1.0 gram) were dissolved in 200 ml. of ether, and the solvent was permitted to evaporate slowly at room temperature. Successive crops of crystals were collected. Crop 1 contained the benzyloxybenzofuran of medicagol (220 mg.). Recrystallization from methanol gave colorless needles, m.p. 159–60° C.

Analysis, calculated for $C_{23}H_{18}O_5$: C, 73.8; H, 4.85; OCH_3, 8.29. Found: C, 74.0; H, 4.96; OCH_3, 8.91.

Crop 2 contained the benzyloxybenzofuran of compound II (V). Recrystallization from methanol provided the analytical sample (300 mg.), m.p. 84° C., which gave no depression with the synthetic 2-(2′-methoxy-4′-benzyloxyphenyl)-6-methoxybenzofuran prepared as described below. The PMR, UV, and IR spectra were also identical.

Analysis, calculated for $C_{23}H_{20}O_4$: C, 76.6; H, 5.56; OCH_3, 17.2. Found: C, 76 5; H, 5.68; OCH_3, 16.9.

2 - (2′ - Methoxy - 4′ - benzyloxyphenyl) - 6 - methoxybenzofuran-3-carboxylic Acid Methyl Ester (IV, R = CH_3). 7-*O*-Benzylcoumestrol acetate (III) (600 mg.), prepared by the method of Jurd (*3*), was converted to its methoxycinnamate derivative by methylative ring opening following the same procedure described above. Recrystallization from methanol gave colorless needles (400 mg.), m.p. 123–25° C.

Analysis, calculated for $C_{25}H_{22}O_6$: C, 71.8; H, 5.26; OCH_3, 22.2. Found: C, 71.8; H, 5.40; OCH_3, 22.1.

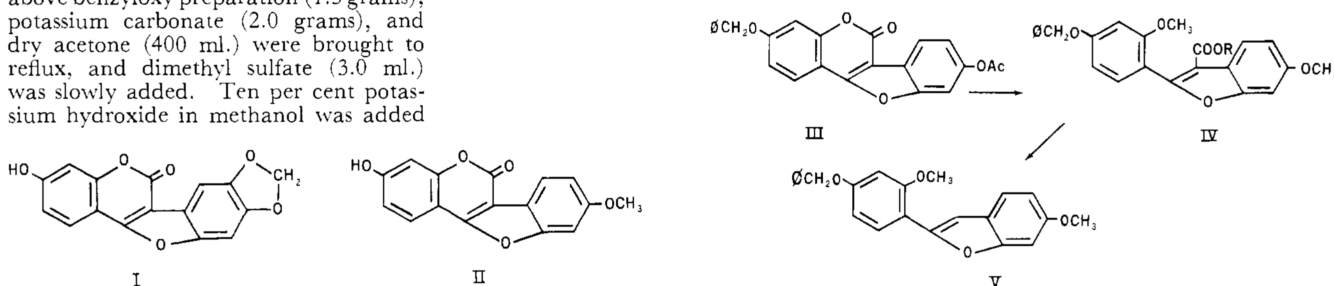
2 - (2′ - Methoxy - 4′ - benzyloxyphenyl) - 6 - methoxybenzofuran - 3-carboxylic Acid (IV, R = H). The above methyl ester (280 mg.) was hydrolyzed in dilute alkali following the same procedure described above. Recrystallization from methanol gave needles (220 mg.), m.p. 207–08° C.

Analysis, calculated for $C_{24}H_{20}O_6$: C, 71.3; H, 4.95; OCH_3, 15.4. Found: C, 71.1; H, 5.05; OCH_3, 15.7.

2 - (2′ - Methoxy - 4′ - benzyloxyphenyl) - 6 - methoxybenzofuran (V). The above acid (190 mg.) was decarboxylated by heating at 210–25° C. for 1 hour. It was purified following the procedure described above. Recrystallization from methanol gave needles (130 mg.), m.p. 84° C.

Analysis, calculated for $C_{23}H_{20}O_4$: C, 76.6; H, 5.56; OCH_3, 17.2. Found: C, 76.6; H, 5.64; OCH_3, 17.0.

Acid Hydrolysis of the Natural Mixture of Medicagol and 4′-*O*-Methylcoumestrol. The mixed acetates of 4′-*O*-methylcoumestrol and medicagol (500 mg.) were hydrolyzed with 86% sulfuric acid (15 ml.) for 15 minutes at room temperature. The mixture was poured into cold 10% potassium hydroxide (150 ml.), and the pH of the solution was adjusted to 4–5 with additional alkali. The mixture was extracted with three 250-ml. portions of ether. The ether phase was dried (sodium sulfate) and concentrated to dryness. The solids were purified by CCD employing a solvent system composed of acetone–ethanol–ether–Skellysolve B–water (10:5:10:2:1). After 175 transfers, the distribution was evaluated by TLC on silica gel G. Fraction 1 (tubes 5 to 25 in the instrument) contained a material which had the same R_f and fluorescence as the hydrolysis product of medicagol, 7,11,12-trihydroxycoumestan (*4*). Fraction 2 (tubes 1 to 49 in the fraction collector)



contained in impure bluish fluorescent compound having the same R_f (0.7) by TLC as 4'-*O*-methylcoumestrol. The contents of the tubes were combined, and the solvent was removed under vacuum. The solids were further purified by column chromatography on silica gel (Davidson). Washing of the column with 8% acetone in Skellysolve B gave pure 4'-*O*-methylcoumestrol. Recrystallization from methanol–acetone (1–1) gave colorless crystals (61 mg.), m.p. 331–32° C., no depression with an authentic sample of 4'-*O*-methylcoumestrol. The PMR, UV, and IR spectra of the isolated and authentic samples were also identical.

Proportion of Medicagol and 4'-*O*-Methylcoumestrol in Mixture. The per cent methoxyl calculated for 4'-*O*-methylcoumestrol is 11.0; the per cent found in the mixture is 8.29. The percentage found can be accounted for if the mixture contains 75.4% of 4'-*O*-methylcoumestrol. Similar calculations on the mixture of the acetates of the two compounds indicate 77.8% 4'-*O*-methylcoumestrol based on 7.45% methoxyl in the mixture. Therefore, the alfalfa plant apparently contains about three times as much 4'-*O*-methylcoumestrol as medicagol.

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ISOLATION AND IDENTIFICATION

Constituents of the Cotton Bud. Terpene Hydrocarbons

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The terpene hydrocarbons from the buds (squares) of Deltapine Smoothleaf cotton were obtained by steam distillation and column chromatography. Components identified and their percentages of the essential oil were: *l*-α-pinene (8.88), *l*-camphene (0.41), β-pinene (1.03), myrcene (8.16), α-phellandrene (0.07), α-terpinene (0.02), *l*-limonene (1.21), β-phellandrene (0.09), *trans*-β-ocimene (3.90), γ-terpinene (trace), and terpinolene (0.21). Various gas chromatographic techniques were utilized for preliminary identifications. Positive identifications were made by infrared, proton resonance, and ultraviolet spectroscopy.

PLANT chemical constitution is of fundamental importance in understanding host plant–insect interrelationships. Compounds elaborated by plants as wastes, metabolic intermediates, defense mechanisms, or insect attractants influence the development of highly specific plant-insect ecologies (*3*). Such a specialized relationship exists between the boll weevil, *Anthonomous grandis* Boheman, and its preferred host, the cotton plant, *Gossypium*. Keller and coworkers (*4*) have presented strong evidence for the existence in cotton of a plant attractant for the boll weevil. A previous claim (*16*) of weevil attractancy by a cotton plant distillate has been made, but was not backed by experimental evidence.

Little is known of the volatile components of the cotton plant, one or more of which presumably are responsible for such attractancy. Power and Chesnut (*10*) steam distilled 3.5 tons of cotton plants, isolated the essential oils by extraction, and ultimately identified trimethylamine, ammonia, acetaldehyde, methanol, and amyl alcohol after extended distillation and extraction procedures. They reported the presence of several unidentified sesquiterpenes. In another examination of the plant for nonvolatile constituents, Power and Chesnut (*11*) isolated dipentene and an unidentified sesquiterpene. Isolation procedures utilized in both of these studies were so severe that considerable isomerization and degradation of the labile terpenes must have occurred. No other reports of work on cotton plant volatiles are known to us, although there are many reports on the nonvolatiles. This work is one of a series of investigations by this laboratory into the nature of the chemical components in the cotton flower bud (*15*). All components are being assayed by entomologists to determine whether they are attractive or repellent to insects.

Experimental

Apparatus. Analytical gas chromatograph: Aerograph HiFi Model 600-C equipped with hydrogen flame detector. Preparative gas chromatograph: Aerograph Autoprep Model A-700 equipped with thermal conductivity detector. Columns, packings, and conditions are listed in Table I.

Beckman - IR5A infrared spectrophotometer.

Beckman DK-2A ratio recording spectrophotometer; matched silica cells of 1.0-cm. path length.

Varian Model A-60 Analytical NMR spectrometer; all spectra run in spectrograde CCl_4.

Kern Full-Circle polarimeter equipped with a 1.0-dm. cell and sodium emission lamp (SLA-5C; George W. Gates and Co.).

Isolation of Cotton Square Extract. *Gossypium hirsutum*, Deltapine Smoothleaf cotton squares (flower buds) were weighed, ground in a SerVall Omnimixer in a minimum of water, and steam distilled in an all-glass system for 1 hour. The distillate was collected in a trap consisting of two flasks connected in series, the first cooled in ice water and the second in an acetone–dry ice bath.