Œstrogenic Activity of Some Derivatives of iso*Flaven and* iso*Flavanol.*

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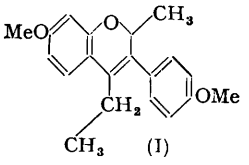
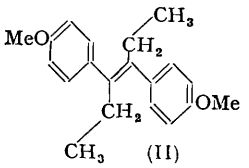
Some new alkyl and phenyl derivatives of *iso*flaven and *iso*flavanol are described. 4-Ethyl-7 : 4′-dimethoxy-2-methyl*iso*flav-3-en, which has the carbon skeleton of stilbœstrol dimethyl ether has œstrogenic activity of the same order, and 7 : 4′-dimethoxy-4-phenyl*iso*flavan-4-ol is also highly active.

THE study of the œstrogenic properties of *iso*flavones and their derivatives was initiated by Bradbury and White (*J.*, 1951, 3447), who isolated genistein (5 : 7 : 4′-trihydroxy*iso*flavone) from a subterranean clover which had been found to be responsible for a sudden outbreak of infertility in sheep in Western Australia. They subsequently described (*J.*, 1953, 871) the properties of several 2-alkyl*iso*flavones and certain 2- and 4-alkyl*iso*flavens. They found that in 7 : 4′-dimethoxy*iso*flav-3-en, itself inactive, introduction of a 4-methyl group conferred activity, whilst the 2-methyl and 4-ethyl compounds were five times as potent as the 4-methyl compound.

The 4-ethyl-2-methyl compound (I), which has the same carbon skeleton as stilbœstrol dimethyl ether, has now been synthesised, and found to have œstrogenic activity of



(I)



(II)

the same order as that of stilbœstrol dimethyl ether (II). Unfortunately, no method has been found for demethylation of this compound without disruption of the molecule. Investigation of the other alkyl derivatives has been prevented by the very low yield (5—8%) of *iso*flavanone obtained when hydrogenating 2-alkyl-methoxy*iso*flavones, most of the products being oily inseparable mixtures. These 2-alkyl-methoxy*iso*flavones with alkyl- or aryl-magnesium halides gave only inseparable mixtures from which no crystalline compound has, as yet, been isolated, whereas the methoxy*iso*flavones react in the normal manner.

In view of the action of certain derivatives of triphenylethylene (Schönberg, Robson, Tadros, and Fahim, *J.*, 1940, 1327) syntheses of a number of phenyl derivatives of *iso*flavanol and *iso*flaven were begun. Whilst this work was in progress Bradbury (*Austral. J. Chem.*, 1953, **6**, 447) described 7 : 4′-dimethoxy- and 5 : 7 : 4′-trimethoxy-4-phenyl-*iso*flav-3-en and their œstrogenic activity in mice. These compounds were prepared, with others, and their activity, as determined in this Institute by the method of Allan, Dickens, and Dodds (*J. Physiol.*, 1930, **68**, 348) using ovariectomised rats, proved to be much less than that of the *iso*flavanols from which they are derived. 7 : 4′-Dimethoxy-4-phenyl*iso*flavan-4-ol has activity approaching that of stilbœstrol dimethyl ether, but introduction of a third methoxyl group in the 5-position reduces this to one-hundredth.

The following Table shows the results of biological tests on these substances compared with tests on free *iso*flavones and on stilbœstrol and its dimethyl ether. (The tests were carried out on the crystalline racemates of unknown configuration isolated from the mixtures of possible stereoisomeric forms resulting from the Grignard reaction.)

Compound	Œstrogenic activity: active at mg. per rat	inactive at mg. per rat
iso*Flavones.*		
7 : 4′-Dihydroxy	—	50
5 : 7 : 4′-Trihydroxy	50	—
7-Glucosidyloxy-5 : 4′-dihydroxy [1]	—	80
iso*Flavan*-4-*ols.*		
7 : 4′-Dimethoxy-2 : 4-diethyl	—	10
7 : 4′-Dimethoxy-4-phenyl	0·03	—
5 : 7 : 4′-Trimethoxy-2 : 4-diethyl	—	10
5 : 7 : 4′-Trimethoxy-4-phenyl	3·0	—
5 : 7 : 4′-Trimethoxy-2-methyl-4-phenyl	—	2
5 : 7 : 4′-Trimethoxy-2 : 4-diphenyl	—	10
iso*Flav*-3-*ens.*		
7 : 4′-Dimethoxy-4-methyl [2]	—	20
7 : 4′-Dimethoxy-4-ethyl [2]	1·0	—
7 : 4′-Dimethoxy-4-phenyl [3]	1·0	—
7 : 4′-Dimethoxy-4-ethyl-2-methyl	0·02	—
5 : 7 : 4′-Trimethoxy-4-ethyl	—	10
5 : 7 : 4′-Trimethoxy-4-phenyl [3]	10·0	—
Stilbœstrol	0·0004	—
Stilbœstrol dimethyl ether	0·020	—

[1] Zemplén and Farkas, *Ber.*, 1943, **76**, 1110. [2] Bradbury and White, *loc. cit.*, 1953. [3] Bradbury, *loc. cit.*

Experimental

Preparation of iso*Flavanols and* iso*Flavens.*—The *iso*flavone or *iso*flavanone was added in portions to ethereal ethyl- or phenyl-magnesium bromide (4 mols.). After addition of dry benzene, the ether was removed by distillation and the benzene solution boiled 6 hr. After being shaken with ice-cold 10% hydrochloric acid, the benzene layer was washed successively with water, 10% aqueous sodium hydroxide, and water, dried, and evaporated. The residue was crystallised from ethanol. The following were prepared by this method :

7 : 4′-Dimethoxy-2-methyl*iso*flavanone (Bradbury and White, *J.*, 1953, 874) gave 4-*ethyl*-7 : 4′-*dimethoxy*-2-*methyl*iso*flav*-3-*en*, needles, m. p. 104—105° (Found : C, 77·5; H, 6·9. $C_{20}H_{22}O_3$ requires C, 77·4; H, 7·1%).

7 : 4′-Dimethoxy*iso*flavone gave 2 : 4-*diethyl*-7 : 4′-*dimethoxy*iso*flavan*-4-*ol*. The fraction distilling at 210—216°/0·25 mm. crystallised in prisms, m. p. 46—47° (Found : C, 73·4; H, 7·8. $C_{21}H_{26}O_4$ requires C, 73·6; H, 7·65%).

Genistein trimethyl ether gave 2 : 4-*diethyl*-5 : 7 : 4′-*trimethoxy*iso*flavan*-4-*ol*, prisms, m. p. 80° (Found : C, 71·0; H, 7·5. $C_{22}H_{28}O_5$ requires C, 70·9; H, 7·6%). Attempts to dehydrate this compound at 200° *in vacuo*, or by prolonged boiling in acetic acid solution were unsuccessful. Boiling with acetic anhydride produced the *acetate*, plates, m. p. 109—110° (Found : C, 69·5; H, 7·4. $C_{24}H_{30}O_6$ requires C, 69·5; H, 7·3%).

Genistein trimethyl ether with phenylmagnesium bromide gave 5 : 7 : 4′-*trimethoxy*-2 : 4-*diphenyl*iso*flavan*-4-*ol*, prisms (from ethanol–ethyl acetate), m. p. 172—173° (Found : C, 76·7; H, 5·75. $C_{30}H_{28}O_5$ requires C, 76·9; H, 6·0%) [*acetate*, m. p. 142—143° (Found : C, 75·0; H, 6·1. $C_{32}H_{30}O_6$ requires C, 75·3; H, 5·9%)].

From 5 : 7 : 4′-trimethoxy*iso*flavanone (Bradbury and White, *loc. cit.*, 1953) were obtained 4-*ethyl*-5 : 7 : 4′-*trimethoxy*iso*flav*-3-*en*, m. p. 107—107·5° (Found : C, 73·6; H, 7·1. $C_{20}H_{22}O_4$ requires C, 73·6; H, 7·1%), and 5 : 7 : 4′-*trimethoxy*-4-*phenyl*iso*flavan*-4-*ol*, needles (from ethanol–ethyl acetate), m. p. 168° (Found : C, 73·1; H, 6·1. $C_{24}H_{24}O_5$ requires C, 73·45; H, 6·2%). This *iso*flavanol was unchanged after being heated at 250°/0·1 mm. for 30 min. but was dehydrated by boiling acetic acid in 1 hr. The product, crystallised from alcohol, had m. p. 157° as Bradbury's 5 : 7 : 4′-trimethoxy-4-phenyl*iso*flav-3-en (Found : C, 77·2; H, 5·9. Calc. for $C_{24}H_{22}O_4$: C, 77·0; H, 5·9%).

7 : 4′-*Dimethoxy*-4-*phenyl*iso*flavan*-4-*ol*.—Bradbury's method for preparation of the corresponding *iso*flaven was followed. The residue from evaporation of the benzene solution separated slowly from ethanol, giving the iso*flavanol* in prisms, m. p. 125—126°, after recrystallisation

from ethanol (Found: C, 76·4; H, 6·1. $C_{23}H_{22}O_4$ requires C, 76·2; H, 6·1%). The mother-liquor and washings were evaporated to small volume and slowly yielded colourless needles, m. p. 132—137°, finally raised by crystallisation from ethanol–ethyl acetate to 138° (Bradbury gives 142°). This compound proved to be 7 : 4′-dimethoxy-4-phenyl*iso*flav-3-en (Found: C, 80·1; H, 5·9. Calc. for $C_{23}H_{20}O_3$: C, 80·2; H, 5·85%).

5 : 7 : 4′-*Trimethoxy*-2-*methyl*iso*flavanone*.—5 : 7 : 4′-Trimethoxy-2-methyl*iso*flavone (2·5 g.), platinum oxide (0·2 g.), and glacial acetic acid (100 ml.) were shaken with hydrogen for 0·5 hr., 1·5 mols. being absorbed. The residue, after removal of catalyst and acetic acid, produced a small yield of crystals from ethanol; these on recrystallisation from the same solvent gave the *iso*flavanone as thin needles, m. p. 194—195° (Found: C, 69·6; H, 6·4. $C_{19}H_{20}O_5$ requires C, 69·5; H, 6·1%).

5 : 7 : 4′-*Trimethoxy*-2-*methyl*-4-*phenyl*iso*flav*-3-*en* was prepared by addition of 5 : 7 : 4′-trimethoxy-2-methyl*iso*flavanone to ethereal phenylmagnesium bromide. The product, isolated in the usual manner, crystallised from ethanol in needles, m. p. 136—137° (Found: C, 73·3; H, 6·5. $C_{25}H_{26}O_5$ requires C, 73·9; H, 6·45%).

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