Coumestrol, Plant Phenolics, and Synthetic Estrogens: a Correlation of Structure and Activity

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A correlation of structure with estrogenic activity of plant phenolics (including coumestrol, isoflavones and desoxybenzoins) and certain synthetic estrogens has been made. Pertinent compounds were re-evaluated under standardized conditions. The most active of the compounds synthesized for this study was 7,4′-diacetoxy-2-methyl-4-ethyl-Δ^3-isoflaven.

Current interest in this laboratory regarding the biological activity of coumestrol and other plant estrogens as compared to the growth-promoting effect of diethylstilbestrol has led to a continued[2] investigation of the structure–activity relationship of certain coumarins, isoflavones, desoxybenzoins and related compounds. In addition, the estrogenic activity of representative samples from these different classes of compounds has been re-evaluated and these activities correlated under identical bioassay conditions.

The activity of the compounds was evaluated by linearly plotting the mean mouse uterine weight for a series of doses. The *dose level* recorded in the Tables represents the quantity of material fed to produce a 25-mg. uterine response as compare to a uterine weight of 10 mg. for untreated animals.[3a,b]

Certain derivatives of diethylstilbestrol and coumestrol have been bioassayed for comparison purposes and the values included in Tables I and II.

Some isoflavens (Table III) are extremely potent estrogens[4]

(1) A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany, California.

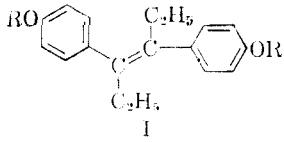
(2) E. M. Bickoff, A. L. Livingston, and A. N. Booth, *Arch. Biochem. Biophys.*, **88**, 262 (1960).

(3) (a) E. M. Bickoff, A. N. Booth, A. L. Livingston, A. P. Hendrickson, and R. L. Lyman, *J. Animal Sci.*, **18**, 1000 (1959); (b) E. M. Bickoff, A. L. Livingston, A. P. Hendrickson, and A. N. Booth, *J. Agr. and Food Chem.*, in press.

(4) W. Lawson, *J. Chem. Soc.*, 4448 (1954).

TABLE I

ESTROGENIC ACTIVITY OF DIETHYLSTILBESTROL DERIVATIVES



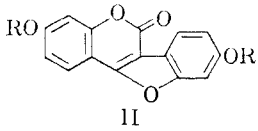
I

No.	R	Dose level, mg.
a	H—[a]	0.00008
b	CH_3CO—[b]	0.00010
c	$CH_3(CH_2)_{14}CO$—[c]	0.00021
d	CH_3—[c]	0.00045

[a] California Corp. for Biochemical Research. [b] E. C. Dodds *et al.*, *Proc. Royal Soc.* (*London*), **127B** 140 (1939). [c] Aldrich Chemical Co.

TABLE II

ESTROGENIC ACTIVITY OF COUMESTROL DERIVATIVES



II

No.	R	Dose level, mg.
a	H—[a]	0.25
b	CH_3CO—[a]	0.33

[a] O. H. Emerson and E. M. Bickoff, *J. Am. Chem. Soc.*, **80**, 4381 (1958).

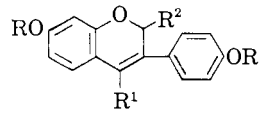
comparable in activity to derivatives of diethylstilbestrol and to the plant estrogen miroestrol.[5] The isoflavens IIIa and IIIb are of special interest since they can be related to diethylstilbestrol through the carbon skeleton[4] and to miroestrol by biogenetic dissection.[6] In turn, as Taylor *et al.*[6] have indicated, this dissection interrelates the plant estrogens, for example, the isoflavones (Table IV). Included in this category of related compounds are certain of the coumarins (Table V) as well as coumestrol and its derivatives.

We then have three classes of related estrogens which can be

(5) D. G. Bounds and G. S. Pope, *J. Chem. Soc.*, 3696 (1960).
(6) N. E. Taylor, D. C. Hodgkin, and J. S. Rollett, *ibid.*, 3685 (1960).

TABLE III

ESTROGENIC ACTIVITY OF RELATED Δ^3-ISOFLAVENS



III

No.	R	R^1	R^2	Dose level, mg.
a	CH_3CO—	C_2H_5—	CH_3—	0.00014
b	CH_3—[a]	C_2H_5—	CH_3—	0.00038
c	CH_3CO—	H—	CH_3—	0.031
d	CH_3—[a]	H—	CH_3—	0.051
e	CH_3—[a]	C_6H_5—	H—	0.005
f	CH_3CO—	C_2H_5—	H—	0.012
g	CH_3—[a]	C_2H_5—	H—	0.060
h	CH_3—[a]	CH_3—	H—	0.208

[a] We are indebted to Mr. W. Lawson for samples of the 7,4′-dimethyl ether derivatives (b, d, e, g and h; also VIIa).

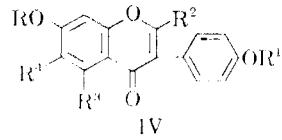
classified according to their biological potency: (1) The most active group includes the naturally occuring miroestrol and certain of the synthetic Δ^3-isoflavens (*e.g.*, IIIa and IIIb) and diethylstilbestrol derivatives. (2) Coumestrol derivatives constitute an intermediate class. (3) The numerous isoflavones and certain coumarins (excluding the 4-alkylcoumarins which will be considered separately) make up the weakest class.

For these studies the common practice of using methyl ether derivatives, which are readily available, has been supplemented whenever possible by the free phenol or the corresponding esters because the ethers tend not only to decrease markedly the biological activity but to do so in an irregular manner. Moreover, demethylation frequently is accomplished by destruction of the molecule, particularly in the case of isoflavanones and isoflavens. An example of this deals with the case of the very active estrogen first prepared by Lawson,[4] 7,4′-dimethoxy-2-methyl-4-ethyl-Δ^3-isoflaven (IIIb) which he could not convert into the desired phenol. We have now prepared the corresponding 7,4′-diacetate (IIIa) by the reaction of ethylmagnesium bromide on 7,4′-diacetoxy-2-methylisoflavanone[7] (VIc).

(7) R. B. Bradbury and D. E. White, *J. Chem. Soc.*, 871 (1953).

TABLE IV

ESTROGENIC ACTIVITY OF RELATED ISOFLAVONES



IV

No.		R	R^1	R^2	R^3	R^4	Dose level, mg.
a		H—[a]	H—	CH_3—	H—	H—	21[b]
b		CH_3CO—[a]	CH_3CO—	CH_3—	H—	H—	. .
c	Formononetin	H—[c]	CH_3—	H—	H—	H—	31
d	Daidzein	H—[c]	H—	H—	H—	H—	11
e	Genistein	H—[c]	H—	H—	HO—	H—	8
f	Biochanin-A	H—[c]	CH_3—	H—	HO—	H—	18
g	Castanin[d,e]	H—	CH_3—	H—	H—	CH_3O—	(15)[f]

[a] See ref. 7. [b] Lespagnol *et al.* (ref. 22) reported this compound (IVa) inactive at the level tested (1 mg.). [c] See ref. 3b. [d] J. J. H. Simes and R. A. Eade, "Australian Phytochemical Survey. III. Saponins in Eastern Australian Flowering Plants," p. 29 (1959). We are indebted to Dr. J. J. H. Simes for a sample of castanin. [e] 7-Hydroxy-6,4′-dimethoxyisoflavone (IVg) has been described as afromosia [T. B. H. McMurry and C. Y. Theng, *J. Chem. Soc.*, 1491 (1960)]. [f] Dose level in parentheses means that the compound was inactive at the indicated level.

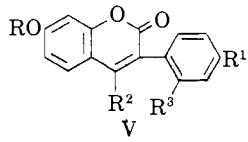
7,4′-Diacetoxy-4-ethyl-Δ^3-isoflaven (IIIf) has also been synthesized in a similar manner.

Some difficulty was encountered in preparing intermediates for these studies. In particular, the hydrogenation of 2-methylisoflavones[7] affords complex mixtures, low yields of 2-methylisoflavanones, and results that are difficult to reproduce. Several different reduction systems were investigated but none was superior to the use of platinum in acetic acid.[4,8] Unsuccessful attempts were made to hydrogenate 7,4′-dihydroxy-2-methylisoflavone (IVa) at 25° (75–150 mm.) in ethanol with 5% palladium-on-charcoal (also at 50°), ethanol–acetic acid 1:1 with platinum oxide, and 0.5 *N* sodium hydroxide with palladium charcoal.[9] Reaction with sodium metabisulfite[10] in sodium hydroxide solution also failed to yield VId.

(8) E. L. Anderson and G. F. Marrian, *J. Biol. Chem.*, **127**, 649 (1939).
(9) E. Breitner, E. Roginski, and P. N. Rylander, *J. Org. Chem.*, **24**, 1855 (1959).
(10) N. Narasimhachari and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **35A**, 202 (1952).

TABLE V

ESTROGENIC ACTIVITY OF RELATED COUMARINS



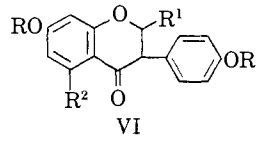
V

No.	R	R^1	R^2	R^3	Dose level, mg.
a	H—	HO—	H—	H—	37
b	CH_3CO—	CH_3CO_2—	H—	H—	40
c	CH_3—[a]	CH_3O—	H—	H—	(50)[b]
d	CH_3CO—	CH_3CO_2—	CH_3CO_2—	H—	(50)[b]
e	H—	HO—	HO—	H—	(40)[b]
f	H—	CH_3O—	HO—	H—	(50)[b]
g	H—	CH_3O—	HO—	CH_3O—	(25)[b]
h	H—	CH_3O—	n-C_3H_7—	H—	0.031
i	CH_3—	CH_3O—	n-C_3H_7—	H—	0.032
j	H—	HO—	n-C_3H_7—	H—	0.10
k	H—	HO—	C_2H_5—	H—	>(0.10)[c]
l	H—	H—	C_2H_5—	H—	0.8
m	H—	CH_3O—	CH_3—	H—	0.2[d]

[a] See ref. 31. [b] Footnote *b*, Table IV. [c] Only slightly active at this level. Because of limited quantities of material higher dosage levels were not tested. [d] Because of limited quantities of material this value was obtained with fewer evaluations.

TABLE VI

ESTROGENIC ACTIVITY OF RELATED ISOFLAVANONES

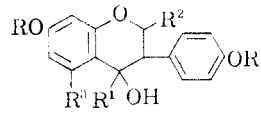


VI

No.	R	R^1	R^2	Dose level, mg.
a	CH_3CO—	H—	H—	25
b	H—	H—	H—	..
c	CH_3CO—	CH_3—	H—	..
d	H—	CH_3—	H—	..
e	CH_3CO—	H—	CH_3CO_2—	18

TABLE VII

ESTROGENIC ACTIVITY OF RELATED ISOFLAVAN-4-OLS



VII

No.	R	R^1	R^2	R^3	Dose level, mg.
a	CH_3—[a]	C_6H_5—	H—	H—	0.0035
b	CH_3CO—	H—	CH_3—	H—	0.320

[a] Footnote *a*, Table III.

The corresponding 7,4′-diacetate[7] (IVb) did not lend itself to hydrogenation with rhodium–alumina in acetic acid or to the Birch reaction[11] (lithium in liquid ammonia with 1,2-dimethoxyethane as solvent).

If the reaction with platinum in acetic acid is allowed to continue until two equivalents of hydrogens are consumed, the major products isolated are 7,4′-diacetoxy-2-methylisoflavan-4-ol[7](VIIb) and 7,4′-diacetoxy-2-methylisoflavanone[7] (VIc) with no sign of starting isoflavone (IVb). The possibility exists that mild oxidation of such a mixture would offer an improved route to the desired ketone (VIc). Such a reaction was attempted with active manganese dioxide[12] in a chloroform solution on a pure sample of 2-methylisoflavanol (VIIb) and afforded a product (an oil which has resisted attempts at crystallization) that had ultraviolet, infrared and chromatographic properties closely resembling those of VIc.

7,4′-Diacetoxy-2-methyl-Δ^3-isoflaven (IIIc) was synthesized in good yield by the action of potassium bisulfate (or other dehydrating agents) on 7,4′-diacetoxy-2-methylisoflavan-4-ol (VIIb).

7,4′-Diacetoxy-2-methylisoflavan-4-ol (VIIb) has been bioassayed and found to be as active[13] as the naturally occurring plant estrogen coumestrol[2,14] (see above). Perhaps the activity of VIIb should be attributed to an *in vivo* dehydration product, such as 7,4′-diacetoxy-2-methyl-Δ^3-isoflaven (IIIc), rather than to the parent alcohol, since

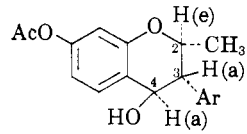
(11) A. J. Birch and H. Smith, *Quart. Revs.*, **12**, 17 (1958).

(12) O. Manecra, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).

(13) Bradbury and White[7] reported that 7-acetoxy-4′-methoxy-2-methylisoflavan-4-ol was inactive at 3.1 mg. per mouse by injection.

(14) E. M. Bickoff, R. L. Lyman, A. L. Livingston, and A. N. Booth, *J. Am. Chem. Soc.*, **80**, 3969 (1958).

we know that the change from VIIb to IIIc results in a tenfold increase of estrogenic activity. The work of Lawson[4] and of Bradbury and White[7] adds to our picture of the isoflavan-4-ols and their products of dehydration. These alcohols are the reaction products from a Grignard reagent on an isoflavanone (*e.g.*, IIIa,b,e,f,g,h; VIIa), from catalytic hydrogenation (VIIb), or lithium aluminum hydride reduction[7] (IIId) of an isoflavone. Since these compounds are estrogenically active and are dehydrated readily, they can be related to VIIb discussed above. Some information concerning the relationship of the groups on the heterocyclic ring of 7,4′-diacetoxy-2-methylisoflavan-4-ol (VIIb) has been obtained by nuclear magnetic resonance studies. The proton spectra of VIIb in $CDCl_3$ was taken at 60 Mc. using a Varian DP 60 spectrometer system.[15] From the splitting of the 2, 3, and 4 proton resonances, the various spin couplings were determined. $J_{2,3}$ was found to be 2.1 cps, and $J_{3,4}$ 7.1 cps. Assuming a pseudo-cyclohexane ring type structure these values indicate an axial–axial configuration for the 3- and 4-protons and an axial-equatorial configuration for the 2- and 3-protons. A constrained methyl group on carbon atom 2 also seems apparent and is reasonable in



the above situation. Apparently *cis* (a,e) elimination of water occurs during the course of converting VIIb to IIIc of a number of compounds which represent various stages of reduction of the plant estrogens; several simple isoflavanones have been isolated (*e.g.*, sophorol,[16] ferreirin and homoferreirin,[17] and padmakastein[10]; some have been synthesized but little mention of their possible estrogenic activity has been made in the literature. In order to test the potency of this family of compounds, 2,3-dihydrodaidzein diacetate (VIa) and 2,3-dihydrogenistein triacetate (VIe) have been synthesized by catalytic hydrogenation of the corresponding isoflavone acetates. Reduction of isoflavones by means of sodium metabisulfite–sodium

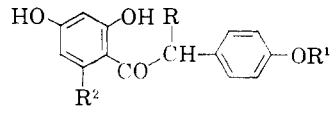
(15) Mention of specific products does not constitute endorsement by the Department of Agriculture over others of a similar nature not mentioned.

(16) H. Suginome, *Tetrahedron Letters*, **19**, 16 (1960).

(17) F. E. King and K. G. Neill, *J. Chem. Soc.*, 4752 (1952).

TABLE VIII

ESTROGENIC ACTIVITY OF RELATED DESOXYBENZOINS



VIII

No.		R	R^1	R^2	Dose level, mg.
a		H—[a]	H—	H—	28
b	Ononetin	H—[b]	CH_3—	H—	(50)[c]
c		H—[d]	H—	HO—	(30)[c]
d	Angolensin	CH_3—[e]	CH_3—	H—	(50)[c]
e		CH_3—	H—	H—	18

[a] See ref. 19. [b] See ref. 21. [c] Practically inactive. [d] See ref. 24. [e] See ref. 20.

hydroxide, as described by Narasimhachari and Seshadri,[10] afforded us *not* the desired dihydro derivatives but rather the desoxybenzoins which are the usual products of alkaline degradation. Thus, 2,4-dihydroxyphenyl-4′-hydroxybenzyl ketone (VIIIa) was obtained from 7,4′-dihydroxy-2-methylisoflavone (IVa), and 2,4,6-trihydroxyphenyl-4′-hydroxybenzyl ketone (VIIIc) was the product from genistein (IVe).[18]

The two dihydro compounds VIa and VIe have been bioassayed and found to impart an estrogenic effect comparable on a weight basis to that of the related isoflavones (Table VI).

The α-alkyl substituted desoxybenzoins as well as the parent ketones have been considered as representing two further stages of reduction of the plant estrogens and selected samples have been bioassayed. While 2,4-dihydroxyphenyl 4′-hydroxybenzyl ketone[19] (VIIIa) as well as 2,4-dihydroxyphenyl 1-(4-hydroxyphenyl)-ethyl ketone[20] (VIIIe) were found to possess biological activity similar

(18) Dr. E. Wong of the Department of Scientific and Industrial Research, Palmerston North, New Zealand, has kindly informed us of similar results with this reaction[10] on certain isoflavones.

(19) E. Walz, *Ann.*, **489**, 118 (1931).

(20) F. E. King, T. J. King, and A. J. Warwick, *J. Chem. Soc.*, 1920 (1952). We are grateful to Dr. T. J. King (Nottingham) for a generous sample of optically active angolensin (VIIId) which, on demethylation, afforded VIIIe as a racemic mixture.

to that of certain of the natural isoflavones, the corresponding 4′-methyl ethers ononetin[21] (VIIIb) and angolensin[20] (VIIId) were practically devoid of activity[22] according to our standards. Considering how relatively weak the angolensin derivatives VIIId and VIIIe were found to be, it would be interesting to reconsider α-ethyldesoxyanisoin which has been reported to be some thousand times more potent[23] (test method not known). 2,4,6-Trihydroxyphenyl 4′-hydroxybenzyl ketone[24] (VIIIc) was of particular interest since it could be considered a primary breakdown product of genistein (IVe), and degradation of IVe with alcoholic potassium hydroxide was reported[25] to increase the estrogenic activity several fold. It was somewhat surprising then to find that Vc was inactive even at high levels.[26] The possibility also exists that the increase in activity reported by Pieterse and Andrews[25] could be attributed to benzil formation. For example, Henne and Bruylants[27] described anisil as active at 0.1 mg. (method of testing not reported) whereas others reported it inactive at the same level.[28]

The report[23] that desoxyanisoin is active at a level several hundred fold lower (*i.e.*, at 0.1 mg.) than we observed for the hydroxyl-substituted desoxybenzoins VIIIa and VIIIc was interesting; however, we found that desoxyanisoin was inactive even at five times the reported dose level.

The potency of the numerous coumarins which have been bioassayed is quite variable (Table V), not only if they are considered as a class of compounds but within specific subseries as well. A number of coumarins closely related to but less potent than the plant estrogen coumestrol have been discussed elsewhere[2] and the importance of the benzofuran portion of the molecule was made quite obvious. Several other coumarins that lack the benzofuran system have been synthesized and included here in order to increase our understanding

(21) W. Baker and F. M. Eastwood, *J. Chem. Soc.*, 2897 (1929).

(22) A. Lespagnol, J. Schmitt, and P. Brunaud, *Bull. soc. chim. France*, 82 (1951), have reported that 2,4-dihydroxydesoxybenzoin was also inactive at the level tested (*i.e.* >1 mg.).

(23) G. Cavallini, M. Goisis, and E. Massarani, *Farm. Sci. e Tec.* (Paris), **3**, 300 (1948); *Chem. Abstr.*, **42**, 8340 (1948).

(24) W. Baker and R. Robinson, *J. Chem. Soc.*, 2713 (1926).

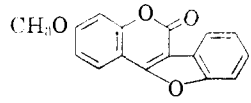
(25) P. J. S. Pieterse and F. N. Andrews, *J. Dairy Sci.*, **39**, 81 (1956).

(26) R. B. Bradbury and D. E. White, *Vitamins and Hormones*, **12**, 207 (1954), reported that 2,4,6-trihydroxyphenyl 4′-hydroxybenzyl ketone was not estrogenic at a dose level of 1.1 mg.

(27) G. Henne and A. Bruylants, *Bull. soc. chim. Belges*, **57**, 320 (1948).

(28) J. A. Hogg and J. Korman, "Medicinal Chemistry," Vol. 2, F. F. Blicke and C. M. Suter, eds., J. Wiley and Sons, Inc., New York, N. Y., 1956, p. 78.

of the minimum requirements within this series. The simplest compounds that still give a positive estrogenic response are 7,4′-dihydroxy-3-phenylcoumarin[30,31] and certain derivatives (Va-c). On the other hand 4,7,4′-trihydroxy-3-phenylcoumarin (Ve) was practically devoid of estrogenic activity, a characteristic typical of all of the 4-hydroxycoumarins that have been tested in this laboratory. It seems possible that the lack of activity within this series can be the consequence of a potentially labile 1,3-carbonyl system (biological detoxification). Although introduction or variation of groups of the 3-(*p*-hydroxphenyl) moiety in general results in a lowering of the activity, there appears to be some allowable variation if this is restricted to the *ortho–para* positions. Such a situation seems to exist in the isoflavone series, since the activities of genistein and the *ortho* analog (5,7,2′-trihydroxyisoflavone) have been reported to differ only by one quarter.[32] The case of 7-methoxybenzofuro-3′,2′,3,4-coumarin[2] (IX) is similar to the above in that the change relative to



IX

7-*O*-methylcoumestrol[2,33] that involves the removal of the *p*-hydroxyl group also results in a potency difference of only one quarter. Although the over-all effect is comparable for the two cases cited above, the reasons may be entirely different. For example, the similarity in the genistein case may be explainable by resonance considerations, whereas the coumestrol relationship may be simply spacial in nature *i.e.*, retention of the rigid planar structure. A number of 4-alkylcoumarins[34a] have been re-examined and compared to other members of this group. In several instances the results (Vh-m) have not agreed

(30) P. R. Bhandari, J. L. Bose, and S. Siddiqui, *J. Sci. Industr. Res.*, **B8**, 189 (1949).

(31) R. B. Bradbury, *Australian J. Chem.*, **7**, 206 (1954).

(32) W. Baker, J. B. Harbone, and W. D. Ollis, *J. Chem. Soc.*, 1860 (1953), have described 5,7,2′-trihydroxyisoflavone as active at 4 mg.; according to Bradbury and White[7] genistein is active at 1 mg. under similar conditions.

(33) L. Jurd, *J. Org. Chem.*, **24**, 1786 (1959).

(34) (a) C. Mentzer, P. Gley, D. Molho, and D. Billet, *Bull. soc. chim. France*, 271 (1946); (b) compounds Vk-l which were among those generously supplied by Professor C. Mentzer have not been described previously. The m.p. (ref.) given in the experimental section are those communicated to us by him.

with previous reports.[34a,35,36] 7,4′-Dihydroxy-4-*n*-propylcoumarin (Vj) was considered the most potent by Mentzer and Gley, but we have found that the mono- and dimethyl ether derivatives (Vh-i) are almost equivalent to one another and are active at only a third the dose level required for the parent phenol(Vj). We have confirmed the observation[35] that, when the 4-alkyl function is varied, maximum activity is realized with the *n*-propyl group. The 4-methyl analog Vm which was considered devoid of activity[35] has also been found to impart an estrogenic response at a quite reasonable level. It also was interesting to learn that while the lack of a 4′-functional group resulted in a decrease of activity, compound Vl was still biologically active and at a fairly low level. Certain features of the structure-activity relationship of the 4-alkylcoumarins, as compared to the other classes of compounds being considered here, seem somewhat anomalous. The most obvious irregularities are the relative activities of the free phenols and their methyl ethers, for the latter are the more potent in this case. Furthermore, since the maximum effect is realized with a 4-*n*-propyl side chain rather than with an ethyl group, one can speculate whether or not these coumarins are truly comparable in their mode and site of action to diethylstilbestrol and the various Δ^3-isoflavens. It would be interesting to know the relative activities of derivatives of 7,4′-dihydroxy-2-methyl-4-propyl-Δ^3-isoflaven.

Experimental[37]

Hydrogenation Experiments.—Hydrogenation was carried out in glacial acetic acid in the presence of prereduced platinum oxide at room temperature and slightly above atmospheric pressure. The experimental data are collected in Table IX and the analytical data are included in Table XI (*vide infra*).

Hydrolysis Reactions.—The conditions for hydrolysis of various compounds are summarized in Table X and the analytical data listed in Table XI.

7,4′-Diacetoxy-2-methyl-Δ^3-isoflaven (IIIc).—A mixture of isoflavanol (VIIb) (50 mg.) and fused potassium bisulfate (150 mg.) was heated in an oil-bath at 150° for 30 min. The product was isolated by extraction with acetone and

(35) P. Gley and C. Mentzer, *Compt. Rend. Soc. Biol.*, **139**, 1055 (1945).

(36) Professor C. Mentzer has kindly informed us that the modified Allen-Doisy bioassay originally used was not very accurate.

(37) (a) Chromatostrip analysis by the method of J. M. Miller and J. G. Kirchner, *Anal. Chem.*, **26**, 2002 (1954). (b) An R_f value is taken as a criterion of purity and implies a single spot on a chromatostrip when detected by ultraviolet light. Unless otherwise stated the solvent system used was ethyl acetate–Skellysolve B (1:1). (c) Preparative scale chromatography was done on silica gel columns (1:35). The solvents employed were Skellysolve B, benzene, and ether.

TABLE IX

HYDROGENATION EXPERIMENTS: CONDITIONS AND PRODUCTS

Reduction conditions				Product(s)	
Compound	Cpd., g.	Solvent, ml.	Catalyst, g.	Equiv. hydrogen	Compound	Wt., g.
IVb	6.8	250	1.0	2	7′4-Diacetoxy-2-methylisoflavan-4-ol[7] (VIIb) and 7,4′-diacetoxy-2-methylisoflavanone (VIc)	0.81[a] 0.15[a]
IVb	1.75	105	0.35	1.4	VIc	0.46[b]
7,4′-Diacetoxyisoflavone[c]	1.0	200	0.08	1	7,4′-Diacetoxyisoflavanone (7,4′-diacetoxy-2,3-dihydrodaidzein) (VIa)	0.76
Triacetate of IVe	0.6	75	0.06	1	5,7,4′-Triacetoxyisoflavanone (5,7,4′-triacetoxy-2,3-dihydrogenistein) (VIe)	0.48

[a] The mixed hydrogenation products were crystallized from alcohol and from benzene–Skellysolve B. This material (2 g.), after chromatography,[37e] afforded VIc and VIIb. [b] After chromatography.[37c] [c] W. Baker, J. Chadderton, J. B. Harborne, and W. D. Ollis, *J. Chem. Soc.*, 1852 (1953).

TABLE X

SUMMARY OF HYDROLYSIS REACTIONS

Compound hydrolyzed	Grams	Solvents and reagent	Ml.	Conditions	Product	Yield, g.
VIa	0.10	Ethanol	10	1 hr.	7,4′-Dihydroxyisoflavanone (VIb)	0.044[a]
		Concd. HCl	1	100°		
Vf	0.40	Acetic acid	4	1 hr. reflux	4,7,4′-Trihydroxy-3-phenylcoumarin (Ve)	0.22
		Acetic anhydride	4			
		47–50% HI	4			

7,4′-Diacetoxy-3-phenylcoumarin[b] (Vb)	2.5	Ethanol Acetone 20% HCl	100 300 20	1.5 hr. 100°	7,4′-Dihydroxy-3-phenylcoumarin (Va)	1.7
7-Hydroxy-4′-methoxy-4-*n*-propylcoumarin (Vh)	0.01	Acetic acid 48% HBr	10 5	2.75 hr. reflux	7,4′-Dihydroxy-4-*n*-propylcoumarin[c] (Vj)	...
Angolensin[d] (VIIId)	3.0	Acetic acid 48% HBr	30 15	2 hr. reflux	2,4-Dihydroxy-1-(4-hydroxyphenyl)-ethyl ketone (VIIIe)	2.2[e]

[a] The yellowish product was purified by filtering through a plug of alumina. [b] Ref. 31. [c] Crystallized from aqueous methanol. [d] M.p. 121–122.5° (rep. 117°),[20] $[\alpha]_D^{25}$ −105° (methanol). [e] The reaction mixture was concentrated *in vacuo* in a water-bath. The red, oily residue was taken up in ether, washed with brine and treated with charcoal.

TABLE XI

ANALYTICAL DATA FOR PRODUCTS LISTED IN TABLES IX AND X

					Analyses, %			
					Calcd.		Found	
Cpd.	M.p., °C.	Recrystn. solvent	R_f	Formula	C	H	C	H
VIIb	140.5–141.5	Benzene–Skellysolve B	0.46	$C_{20}H_{20}O_6$	67.4	5.7	67.5	5.65
VIc	156–157.5	Ethanol	0.72	...	...	...	...	...
VIa	155.5–156	Ethanol	0.71	$C_{19}H_{16}O_6$	67.05	4.75	67.1	4.76
VIe	173–175	Ethanol	0.58	$C_{21}H_{18}O_8$	63.31	4.55	63.3	4.53
VIb	247–249	H_2O/ethanol	0.44	$C_{15}H_{12}O_4$	70.30	4.72	70.3	4.86
Ve	350 dec.	H_2O/ethanol	0.17[b]	$C_{15}H_{10}O_5$	66.67	3.73	66.6	3.79
Va	327–329 dec.[c]	Acetone–methanol	0.44	$C_{15}H_{10}O_4$	70.86	3.96	70.5	4.07
VIIIe[d]	103 (sintering from 75)	Ether–Skelly-solve B	0.58	$C_{15}H_{14}O_4$	69.75	5.56	69.5	5.68

[a] Chromatostrip.[37a,b] [b] R_f 0.70 in abs. ethanol–chloroform 1:3. [c] Reported[30] m.p. 320–321° dec. [d] Lit.[20] m.p. 139–140°. Racemic product (VIIIe) has $[\alpha]_D^{25}$ +0.5° (methanol). Dr. T. J. King has kindly informed us that VIIIe (m.p. 103°) is definitely demethylated, angolensin and the original[20] substance (m.p. 139–140°) are not.

weighed 22 mg. after one crystallization from benzene–Skellysolve B (small granules), m.p. 112–115°, R_f 0.85.

Anal. Calcd. for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36. Found: C, 71.0; H, 5.53.

Dehydration could also be effected by refluxing a solution of VIIb in acetic acid or shaking a benzene solution of the alcohol with phosphorus pentoxide at room temperature.

Attempted Reductions with Sodium Metabisulfite.[10]—**A.** A boiling solution of 7,4′-dihydroxy-2-methylisoflavone (IVa) (100 mg.) and sodium hydroxide (500 mg.) in water (10 ml.) was treated portionwise with 700 mg. of sodium metabisulfite. After 5 min. the solution was cooled, acidified and the precipitate collected (63 mg., m.p. 175–180°). Crystallization from aqueous ethanol afforded 2,4-dihydroxyphenyl 4′-hydroxybenzyl ketone (VIIIa), m.p. and mixture m.p. 185–187°, R_f 0.5.

B. Genistein (IVe) (50 mg.), when allowed to react with sodium metabisulfite (500 mg.), afforded 2,4,6-trihydroxyphenyl 4′-hydroxybenzyl ketone (VIIIe), m.p. 260° dec. (identified by mixture m.p. and chromatostrip behavior).

7,4′-Diacetoxy-2-methyl-4-ethyl-Δ^3-isoflaven (IIIa).—Ethylmagnesium bromide (9.5 mmoles) in 4 ml. of ether was added slowly to a stirred solution of 340 mg. of 7,4′-diacetoxy-2-methylisoflavanone (VIc) in 20 ml. of anhydrous 1,2-dimethoxyethane[38] under nitrogen. A grey precipitate rapidly formed. After the mixture was stirred 5 hr.[39] at room temperature the complex was decomposed with ice and dilute hydrochloric acid, extracted with ether, and worked up in the usual manner; yield, 390 mg., R_f 0.71. The oily product was allowed to react with acetic anhydride (5 ml.) and pyridine (1 ml.) for 2 hr. on the steambath and then treated with ice, and extracted with ether. Chromatography[37c] afforded 75 mg. of solid, m.p. 155–160°. After three recrystallizations from ethanol, the analytical sample of IIIa was obtained as colorless, rectangular plates; yield 31 mg.; m.p. 168–169° (Kofler block); R_f 0.83.

Anal. Calcd. for $C_{22}H_{22}O_5$: C, 72.11; H, 6.05. Found: C, 72.2; H, 6.08.

7,4′-Diacetoxy-4-ethyl-Δ^3-isoflaven (IIIf).—Dihydrodaidzein diacetate (VIa) (500 mg.) was treated exactly as described for IIIa. Chromatography afforded, as the main fraction, a yellow oil (340 mg.) which failed to solidify. After two distillations at 190–200° (20 microns) IIIf was obtained as a colorless glass which was quite hygroscopic and appeared homogeneous on a chromatostrip (R_f 0.87).

Anal. Calcd. for $C_{21}H_2O_{20}.H_2O$: C, 68.09; H, 5.99. Found: C, 68.1; H, 5.82.

4,7-Dihydroxy-4′-methoxy-3-phenylcoumarin (Vf).—A mixture of 2,4-dihydroxyphenyl 4′-methoxybenzyl ketone[21] (VIIIb) (5.0 g.), anhydrous potassium carbonate (15 g.), methyl chloroformate[40] (5 ml.), and acetone (100 ml.) was refluxed for 3 hr. The product was isolated (5.8 g.) and then treated with 1 *N*

(38) It appears advantageous to use 1,2-dimethoxyethane as the solvent for these sparingly soluble compounds in Grignard reactions. It can also be used to an advantage in Birch reactions as well as for borohydride reductions.

(39) Stirring overnight at room temperature did not improve the yield of IIIa. Refluxing the mixture for 6 hr. greatly decreased the yield and purity of the product.

(40) A. H. Gilbert, A. McGookin, and A. Robertson, *J. Chem. Soc.*, 3740 (1957).

sodium hydroxide (250 ml.). for 1 hr. at 65°. Acidification and crystallization from methanol yielded 3.1 g. of Vf as colorless needles (m.p. 274–280°; R_f 0.35). A sample recrystallized for analysis had the same melting point range.

Anal. Calcd. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26. Found: C, 67.8; H, 4.24.

4,7,4′-Triacetoxy-3-phenylcoumarin (Vd) was obtained by boiling a mixture of Ve (700 mg.) and fused sodium acetate (2 g.) in acetic anhydride (20 ml.) for 10 min. The triacetate Vd crystallized from a small volume of methanol as colorless needles (630 mg.), m.p. 189–191° (R_f 0.55).

Anal. Calcd. for $C_{21}H_{16}O_8$: C, 63.63; H, 4.07. Found: C, 63.9; H, 4.04.

4-Alkylcoumarins.[34]

	M.p., °C. (ref.)	M.p., °C. (found)	R_f
Vh	200[34a]	203–204 (Kofler block)	0.67
Vi	156[34a]	153–153.5	0.86
Vj	265[34a]	265 dec.	0.46
Vk	317[34b]	310 dec. (Kofler block)	0.37
Vl	279[34b]	274 (Kofler block)	0.71
Vm	232[34a]	227–230	0.62

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Oxazolocoumarins

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Nine oxazolocoumarins (II) (nitrogen analogs of psoralens) were prepared from 6-amino-7-hydroxy-4,8-dimethylcoumarin. These compounds have been subjected to a number of biological tests. Some of them have been found to be mild central nervous system depressants. One (II, R = CH_3) is active against powdery mildew on cucumbers. None shows psoralen-like activity on skin.

Psoralen (Ia) and its simple substitution products (*e.g.*, Ib, Ic) are highly active in promoting erythema and pigmentation in skin