

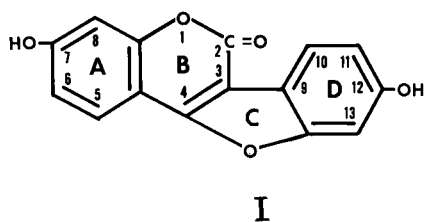
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## Synthesis of a Series of Isomers of Coumestrol

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Three position isomers of the coumestan (2) coumestrol have been synthesized from appropriately substituted flavylum salts. The dimethyl ethers and diacetates of the isomers and several possible monomethyl ethers and their acetates have been prepared.

Coumestans are a relatively new class of naturally occurring compounds, that have been identified in a number of plants (3-6). This class of compounds is of particular interest because of coumestrol (7), found in forage crops (8), which has estrogenic properties (9) and a relationship to pathogenic attack of the plant (10-11). It is the simplest naturally occurring coumestan and is called 7,12-dihydroxycoumestan (I). The only other known naturally occurring disubstituted coumestan is its 12-methoxy derivative (12). All known naturally occurring coumestans are characterized by the substitution of hydroxyl and methoxyl groups at the 7 and 12 positions in addition to other ring positions (1). Tri-substituted coumestans include sativol (13), lucernol (13), trifoliol (3), and psoralidin (6). Wedelolactone (4) and norwedelolactone (14) are the only known naturally occurring tetra-substituted coumestans.



None of the naturally occurring tri- or tetra-substituted coumestans which have been tested have shown any estrogenic activity. However, both 7-methoxy (15) and 12-methoxycoumestrol have estrogenic activity (9). It would be of interest to evaluate the estrogenic activity of other possible disubstituted coumestans. This paper reports the synthesis of the 7,10-; 7,11-; and 7,13-dihydroxycoumestans and a number of their methylated and acetylated derivatives.

Several synthetic approaches have been employed for the synthesis of this class of compounds (15). Jurd's procedure (15) involving the oxidation of an

appropriately substituted flavylum salt to form the coumestan offered the best opportunity to prepare a series of compounds in which the substitution pattern in the D-ring was varied. Naturally occurring coumestans, coumestrol (15,16), medicagol (17), 3-methoxycoumestrol (18), and trifoliol (3), and two synthetic coumestans, 7-methoxy-12-hydroxycoumestan (15) and 7-hydroxy-13-methoxycoumestan (VIIa) (15) have been previously synthesized using this procedure.

An appropriately substituted *ortho*-hydroxybenzaldehyde (II) is condensed in an ethereal hydrogen chloride solution with  $\omega$ -methoxy-2,4-dibenzoyloxyacetophenone (III), to form the benzyloxyflavylum salt (IV). The salt is debenzylated with hydrochloric acid to give the desired flavylum salt (V). The salt is oxidized with hydrogen peroxide to give the 3-carbomethoxybenzofuran (VI) which is then rapidly hydrolyzed and lactonized with sulfuric acid to give the coumestan (VII). The orientation pattern of the D-ring (I) is governed by the aldehyde (II). An aldehyde (II) substituted at  $R_1$  ( $R_2 = R_3 = H$ ) will produce substitution in the 13-position of the D-ring (VII). Similarly,  $R_2$  ( $R_1 = R_3 = H$ ) substituted aldehydes produce the 11-series and  $R_3$  ( $R_1 = R_2 = H$ ), the 10-series. Table I gives the weights of reactants used in the coupling reaction and the weights of crude product obtained from each of the synthetic steps to prepare the crude coumestans. Demethylation of 7-hydroxy-13-methoxycoumestan (VIIa) (15) by fusion with aniline hydrochloride gave 7,13-dihydroxycoumestan (VIIb).

Selective methylation of coumestrol diacetate, with methyl iodide in refluxing acetone, followed by alkaline deacetylation to give the 7-methoxy derivative, has been previously reported (19). The position of the methoxyl group was later confirmed by its unequivocal synthesis (15). Using this procedure, 7-methoxy-10-hydroxycoumestan (VIIc) and 7-methoxy-11-hydroxycoumestan (VIId) were prepared from their diacetates. The acetates and methyl ethers of the coumestans were prepared and their analyses and melting points are summarized in Table II.

TABLE I  
Yield Data on the Several Steps Leading to the Coumestans

Coumestan prepared	A (a) g.	Starting materials B(aldehyde)	g.	Step A	Step B	Step C
				(yield) g.	(yield) g.	(yield) g.
7,10-Dihydroxy-	80.0	$\gamma$ -resorcyaldehyde	35.0	89.3	31.6	15.2
7-Hydroxy-10-methoxy-	12.0	2-hydroxy-6-methoxy-benzaldehyde	4.63	14.5	5.5	2.3
7,11-Dihydroxy-	65.0	2,5-dihydroxy-benzaldehyde	25.0	64.4	29.5	16.4
7-Hydroxy-13-methoxy-	78.0	<i>o</i> -vanillin	30.0	68.0	26.5	11.5

(a) A =  $\omega$ -methoxy-2,4-dibenzyloxyacetophenone.

TABLE II  
Melting Points and Analyses of Acetate and Methyl Ether Derivatives of Coumestans

Coumestan	m. p., °C	Analysis							
		Calculated				Found			
		C %	H %	OCH <sub>3</sub> %	OAc %	C %	H %	OCH <sub>3</sub> %	OAc %
7,10-Dimethoxy-	230-231	68.9	4.08	20.9		69.0	4.25	20.7	
7,10-Diacetyloxy-	247-248	64.8	3.43		24.4	64.8	3.49		24.4
7-Methoxy-10-acetyloxy	236	66.7	3.73	9.56	13.2	66.6	3.75	9.28	13.3
7-Acetyloxy-10-methoxy-	288-289	66.7	3.73	9.56	13.2	65.8	3.73	9.55	13.4
7,11-Dimethoxy-	211.5	68.9	4.08	20.9		69.0	4.20	21.0	
7,11-Diacetyloxy-	253	64.8	3.43		24.4	64.2	3.54		24.6
7-Methoxy-11-acetyloxy-	229-231	66.7	3.73	9.56	13.2	66.5	3.79	9.48	13.3
7,13-Dimethoxy-	204 (a)	68.9	4.08	20.9		68.9	4.12	21.0	
7,13-Diacetyloxy-	214	64.8	3.43		24.4	64.7	3.70		23.3
7-Acetyloxy-13-methoxy-	214-215	66.7	3.73	9.56	13.2	66.9	3.86	9.46	13.0

(a) Lit. (15) m.p. 195°.

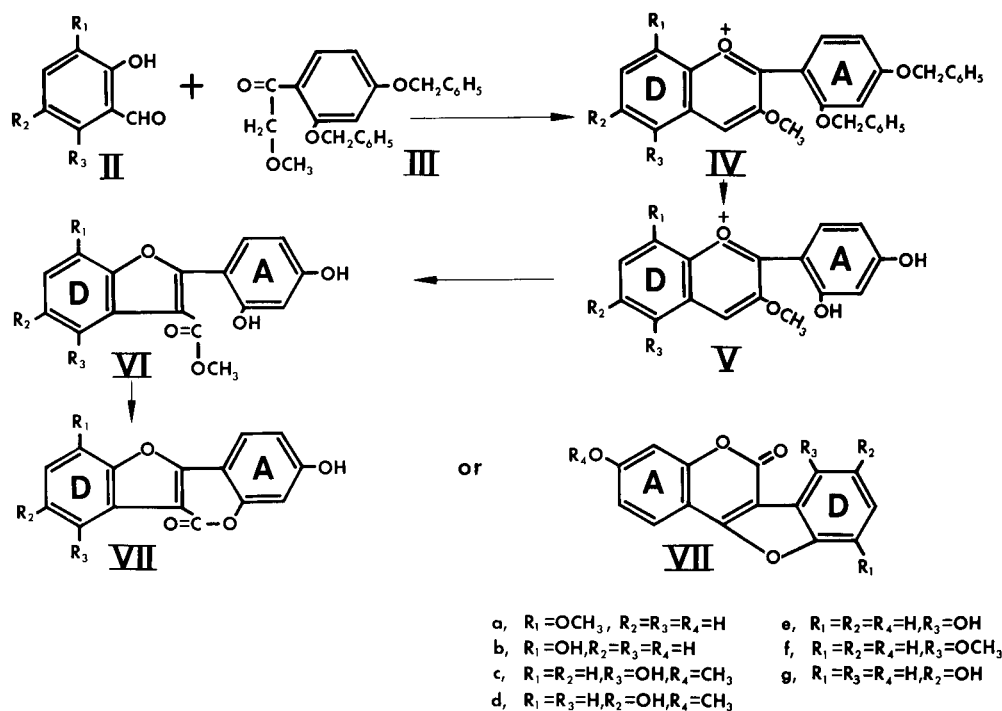
#### EXPERIMENTAL (20)

##### Synthesis of the Coumestans (Table I).

Two of the aldehydes, *ortho*-vanillin, and 2,5-dihydroxybenzaldehyde, were commercial preparations. The other two aldehydes,  $\gamma$ -resorcyaldehyde (21) and 2-hydroxy-6-methoxybenzaldehyde (22) were prepared using literature procedures.

##### Step A. Coupling Reaction.

A solution of  $\omega$ -methoxy-2,4-dibenzyloxyacetophenone and the required aldehyde in a mixture of ether and ethyl acetate was cooled to zero degrees and hydrogen chloride gas was bubbled in for 2 hours. The mixture was left standing at zero degrees overnight. The deep red solids were collected and washed with ether and air-dried. The weights obtained are recorded in Table I.



#### Step B. Debenzylation.

The benzyloxyflavylium salt (IV) was heated on a steam bath for one hour with a 1:1 mixture of glacial acetic acid and concentrated hydrochloric acid. The hot mixture was diluted with 10% aqueous hydrochloric acid, cooled, and layered with benzene/Skellysolve B (1/1). The product was collected and washed several times with boiling 10% methanolic hydrochloric acid.

#### Step C. Oxidation of the Flavylium Salts.

The flavylium salt was suspended in warm methanol and water and treated slowly with 30% aqueous hydrogen peroxide. After 15 minutes the mixture was diluted with water and extracted several times with ether. The ether extracts were combined, dried, and taken to dryness *in vacuo*.

#### Step D. Lactonization to Coumestans (VII).

The crude solids were dissolved in glacial acetic acid and acidified with 50% aqueous sulfuric acid. After heating on a steam bath for 20 minutes a brown solid separated. After drying, the crude coumestan was ready for purification.

The modification of the oxidation procedure described for the preparation of 7-methoxycoumestrol (15) gave the purest product. In the preparation of the dihydroxycoumestans, the solvent volumes used were similar to those reported. However, when preparing methoxycoumestans, higher yields were consistently obtained when the solvent volumes were reduced by 30 to 40%.

#### Purification of Coumestans.

Purification of the crude coumestans obtained from step C, Table I.

##### (a) 7,10-Dihydroxycoumestan (VIIe).

The crude solids were purified by countercurrent distribution (CCD) in a robot operated 200 ml. per tube instrument using methanol/ether/Skellysolve B/water (5/10/10/2) as the solvent system. After 250 transfers, tubes 51-150 off the instrument were combined and taken to dryness to give 8.3 g. of a tan solid. The pure material (3.9 g.) m.p. 278-279°, was obtained after several recrystallizations from tetrahydrofuran.

*Anal.* Calcd. for  $\text{C}_{15}\text{H}_8\text{O}_5$ : C, 67.2; H, 3.01. Found: C, 67.3; H, 3.04.

##### (b) 7-Hydroxy-10-methoxycoumestan (VIIf).

The crude material was purified by repeated recrystallizations from tetrahydrofuran to give 1.6 g. of white needles, m.p. 333-334°.

*Anal.* Calcd. for  $\text{C}_{16}\text{H}_{10}\text{O}_5$ : C, 68.1; H, 3.57;  $\text{OCH}_3$ , 11.0. Found: C, 68.1; H, 3.74;  $\text{OCH}_3$ , 10.9.

##### (c) 7,11-Dihydroxycoumestan (VIIg).

The crude solids were acetylated and the diacetate was recrystallized from chloroform to give 9.45 g. of white needles. A portion (4.0 g.) of the diacetate was deacetylated with  $\frac{1}{2}$ % methanolic potassium hydroxide. The solids obtained were recrystallized from tetrahydrofuran to give 1.02 g. of a white solid, m.p. > 350°.

*Anal.* Calcd. for  $\text{C}_{15}\text{H}_8\text{O}_5$ : C, 67.2; H, 3.01. Found: C, 67.5; H, 3.35.

##### (d) 7-Hydroxy-13-methoxycoumestan (VIIa).

The solids were acetylated as above to give 6.2 g. of white needles. A portion (5.0 g.) of the diacetate was deacetylated and the solids recrystallized from methanol to give 2.98 g. of a white solid, m.p. 303°. The product was identical with an authentic sample of 7-hydroxy-13-methoxycoumestan, m.p. 289° (15).

#### Derivatives of coumestans (Table II).

The coumestans were acetylated in the usual manner with boiling acetic anhydride and sodium acetate. Methylations were run in acetone-potassium carbonate using dimethyl sulfate as the methylating agent.

##### 7,13-Dihydroxycoumestan (VIIb).

A powdered mixture of 7-hydroxy-13-methoxycoumestan (1.58 g.) and aniline hydrochloride (1.6 g.) was heated at 250° for 30 minutes. After cooling to room temperature, the dark solids were washed several times with water and dried to give 1.2 g. of a tan solid. An analytical sample was prepared by several recrystallizations from tetrahydrofuran to give 730 mg. of a white solid, m.p. > 325°.

*Anal.* Calcd. for  $\text{C}_{15}\text{H}_8\text{O}_5$ : C, 67.2; H, 3.01. Found: C, 67.0; H, 3.12.

##### 7-Methoxy-10-hydroxycoumestan (VIIc).

A mixture of 7,10-diacetyloxycoumestan (2.54 g.), anhydrous po-

tassium carbonate (2.5 g.), acetone (175 ml.), and methyl iodide (38 ml.) was heated at reflux for 72 hours. The reaction mixture was cooled, filtered, and the solids washed with water. The remaining solids were combined with the acetone filtrate and taken to dryness *in vacuo*. The crude acetate was hydrolyzed with 1/2% methanolic potassium hydroxide to give 1.02 g. of a white solid. The selective methylation reaction produced a mixture of the product, the dimethyl ether, and the 7,10-dihydroxycoumestan. For purification, the crude solids were chromatographed on a silica gel column (4 x 16 cm.) and developed with increasing amounts of ether in Skellysolve B. The fraction eluted with 5% ether was taken to dryness, giving 350 mg. of a white solid. Recrystallization from acetone gave 210 mg. of a white solid, m.p. 234-235°.

*Anal.* Calcd. for  $C_{18}H_{10}O_5$ : C, 68.1; H, 3.57;  $OCH_3$ , 11.0. Found: C, 68.2; H, 3.62;  $OCH_3$ , 10.9.

#### 7-Methoxy-11-hydroxycoumestan (VIIId).

Using the same procedure as above, a mixture of 7,11-diacetyl-oxy-coumestan (2.0 g.), potassium carbonate (12.0 g.), acetone (140 ml.), and methyl iodide (30 ml.) was heated at reflux for 30 hours. After deacetylation, the crude solids (1.24 g.) were purified by CCD in a robot-operated 20 ml. per tube instrument using acetone/Skellysolve B/ether/methanol/water (10/6/6/4/5) as the solvent system. After 170 transfers, tubes 56-95 in the instrument were combined and taken to dryness. The solids were recrystallized from acetone giving 830 mg. of a white solid, m.p. 281-282.5°.

*Anal.* Calcd. for  $C_{18}H_{10}O_5$ : C, 68.1; H, 3.57;  $OCH_3$ , 11.0. Found: C, 68.3; H, 3.59;  $OCH_3$ , 11.0.

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Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

#### REFERENCES

- (1) A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.
- (2) The trivial name coumestan is used for the skeletal structure of the heterocyclic, four-ring system having the systematic name, 6*H*-benzofuro[3,2,c][1]benzopyrano-6-one (23,24).
- (3) A. L. Livingston, E. M. Bickoff, R. E. Lundin and L. Jurd, *Tetrahedron*, **20**, 1963 (1964).
- (4) T. R. Govindachari, K. Nagarajan and P. C. Parthasarthy, *J. Chem. Soc.*, 548 (1957).
- (5) R. R. Spencer, B. E. Knuckles and E. M. Bickoff, *J. Org. Chem.*, **31**, 988 (1966).
- (6) H. N. Khastgir, P. C. Duttagupta and P. Sengupta, *Tetrahedron*, **14**, 275 (1961).
- (7) E. M. Bickoff, R. L. Lyman, A. L. Livingston and A. N. Booth, *J. Am. Chem. Soc.*, **80**, 3969 (1958).
- (8) R. L. Lyman, E. M. Bickoff, A. N. Booth and A. L. Livingston, *Arch. Biochem. Biophys.*, **80**, 61 (1959).
- (9) E. M. Bickoff, A. L. Livingston and A. N. Booth, *ibid.*, **88**, 262 (1960).
- (10) G. M. Loper and C. H. Hanson, *Crop Sci.*, **4**, 480 (1964).
- (11) G. M. Loper, C. H. Hanson and J. H. Graham, *J. Agronomy*, (in press).
- (12) E. M. Bickoff, A. L. Livingston, S. C. Witt, R. E. Lundin and R. R. Spencer, *J. Agr. and Food Chem.*, **13**, 597 (1965).
- (13) R. R. Spencer, E. M. Bickoff, R. E. Lundin and B. E. Knuckles, *ibid.*, **14**, 162 (1966).
- (14) N. R. Drishnaswamy and T. R. Seshadri, "Naturally-occurring Phenyl-coumarins," in the "Recent Progress in the Chemistry of Natural and Synthetic Colouring Matters and Related Fields," T. S. Gore, Ed., Academic Press, New York and London, p. 235, 1962.
- (15) L. Jurd, *J. Org. Chem.*, **29**, 3036 (1964).
- (16) L. Jurd, *Tetrahedron Letters*, **18**, 1151 (1963).
- (17) L. Jurd, *J. Pharm. Sci.*, **54**, 1221 (1965).
- (18) E. M. Bickoff, R. R. Spencer, B. E. Knuckles and R. E. Lundin, *J. Agr. and Food Chem.*, **14**, 444 (1966).
- (19) L. Jurd, *J. Org. Chem.*, **24**, 1786 (1959).
- (20) Melting points were taken in a capillary tube and are uncorrected.
- (21) J. R. Merchant and A. J. Mountwala, *J. Org. Chem.*, **23**, 1774 (1958).
- (22) L. R. Row and T. R. Seshadri, *Proc. Ind. Acad. Sci.*, **19A**, 141 (1944).
- (23) C. Deschamps-Vallet and C. Mentzer, *Compt. Rend.*, **251**, 736 (1960).
- (24) C. Mentzer, *Mem. Museum Nat.*, (Paris), **D 1 (11): 1-46** (1960).

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