Anal. Calcd for $C_{17}H_{32}O_2$: C, 76.06; H, 12.02. Found: C, 76.32; H, 12.27.

***cis*-5-Dodecen-1-ol Acetate (4a).**—A solution of 7.0 g (0.026 mole) of **3a**, 28 ml of AcOH, and 7.0 ml (0.098 mole) of AcCl was refluxed for 7 hr and then allowed to stand overnight. The solution was poured onto ice diluted to about 200 ml with saturated NaCl and extracted three times with ether. The combined ether layers were washed (three times with H_2O and with 5% Na_2CO_3 and then twice with NaCl), and the ether extract was dried (Na_2SO_4) and distilled to yield 5.4 g (92%) of colorless liquid: bp 78–83° (0.06 mm); $n^{25}D$ 1.4422; ir absorption at 2920 (CH), 1740 and 1230 (primary acetate), and at 720 cm^{-1} (*cis*-CH=CH). A weak band at 965 cm^{-1} represented *trans* double bond impurity. Gas chromatography revealed a single sharp peak, retention time 54 sec at 150° and 40 cc of N_2/min.

Anal. Calcd for $C_{14}H_{26}O_2$: C, 74.28; H, 11.58. Found: C, 74.47; H, 11.45.

***trans*-5-Dodecen-1-ol Acetate (4b).**—Reaction between **3b** and AcCl by the procedure used to obtain **4a** gave the desired product (90%), bp 71–74° (0.05 mm), $n^{25}D$ 1.4419. The ir spectrum was identical with that for **4a** except for a more intense *trans* double bond absorption at 970 cm^{-1} and a less intense double bond absorption at 720 cm^{-1} that represented *cis* impurity. Gas chromatography showed a single sharp peak, retention time of 54 sec at 150° and 55 cc of N_2/min.

Anal. Calcd for $C_{14}H_{26}O$: C, 74.28; H, 11.58. Found: C, 74.43; H, 11.76.

Acknowledgments.—Attractancy tests were conducted at various laboratories of the Entomology Research Division under the supervision of J. W. Balock, Mexico City, Mexico (*A. ludens*); L. F. Steiner, Honolulu, Hawaii (*C. capitata, D. dorsalis, D. cucurbitae*); A. L. Sparks, Tifton, Georgia (*S. frugiperda*); B. A. Butt, Yakima, Washington (*C. pomonella*); A. N. Kishaba, Riverside, California (*T. ni*); M. T. Ouye, Brownsville, Texas (*P. gossypiella*); and by C. W. Collier, Plant Pest Control Division, Falmouth, Massachusetts (*P. dispar*).

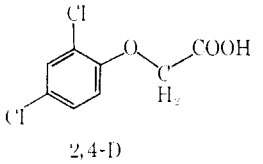
Compounds Related to 2,4-Dichlorophenoxyacetic Acid and Its Derivatives

JORGE R. BARRIO

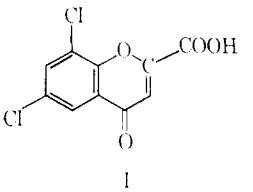
Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

Received September 21, 1967

2,4-Dichlorophenoxyacetic acid (2,4-D) and its derivatives, whose phytohormonal activity is as intense as that of indolebutyric acid or even greater,[1] effectively promote plant growth and also appear to be the most important organic herbicides acting through growth regulation rather than through necrotic or toxic effects.[2] 2-Carboxy-6,8-dichloro-γ-chromone (I) structurally related to 2,4-D and some derivatives have been prepared and tested for phytohormonal activity.[3] There are three differences in 2,4-D and I. One is the presence of the acrylic-type unsaturation; second, there is the presence of the keto group; and third is the fact that in I the molecule should be largely planar and the O–C–CO_2H chain is "tied" down by being part of a



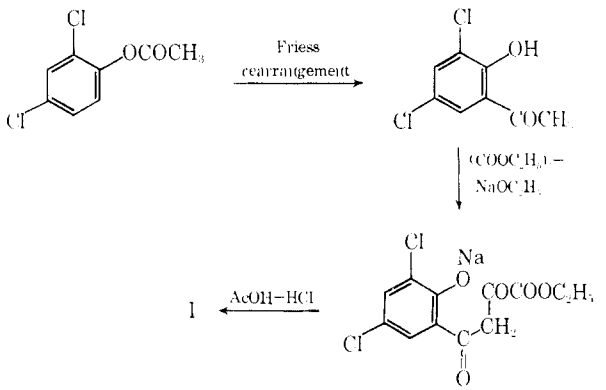
2,4-D



I

ring. In 2,4-D the molecule is not planar and the OCH_2CO_2H is free to rotate. The general process of synthesis is shown in Scheme I.

SCHEME I

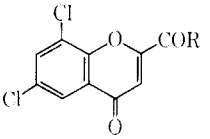


Biological Activity.—Phytoactivity assays of I, V, and VI were carried out on sunflower, wheat, yellow maize, and grain sorghum. I was neutralized with 0.1 *N* NaOH in order to increase its solubility. VI could not be tested because of its insolubility in water.

Tests were performed as follows. (a) For tests on germinating seeds, in Koenig germinators, Hoagland nutrient solution was used with addition of the test substance at a concentration of 20 or 40 mg/100 ml. (b) Germination tests were performed on seeds in the conditions described above, with 24 hr of previous soaking in Hoagland nutrient solution with the addition of the test substance at a concentration of 20 or 40 mg/100 ml. (c) Tests were made on seeds treated as in b, then planted, and the young plants were sprayed with the same solution in aerosol form.

The compounds tested showed no phytohormonal activity. In tests on wheat, slightly less development was seen in the initial growth phase, as compared with controls. However, no significant difference was observed during later development.

TABLE I



Compd	R	Mp, °C	Yield, %	Formula	Analyses
I	OH	254–255	56	$C_{10}H_4Cl_2O_4$	C, H, Cl
II	OC_2H_5	123–124	77	$C_{12}H_8Cl_2O_4$	C, H; Cl[a]
III	$O(CH_2)_2CH_3$	97–98	77	$C_{13}H_{10}Cl_2O_4$	C, H, Cl
IV	$OCH(CH_3)_2$	138–139	82	$C_{13}H_{10}Cl_2O_4$	C, H, Cl
V	$O(CH_2)_5CH_3$	117–118	87	$C_{15}H_{14}Cl_2O_4$	C, H; Cl[b]
VI	$N(C_2H_5)_2$	155–156	61	$C_{14}H_{13}Cl_2NO_3$	C, H, N
VII	Cl	131–132	6	$C_{10}H_3Cl_3O_3$	C, H

[a] Cl: calcd, 24.73; found, 24.20. [b] Cl: calcd, 22.54; found, 22.09.

(1) F. A. Gilbert, *Chem. Rev.*, **39**, 199 (1946).

(2) E. M. Hildebrand, *Science*, **103**, 465 (1946).

(3) Phytohormonal assays were performed by E. Vottero, Facultad de Agronomía y Veterinaria, Universidad de Buenos Aires.

Experimental Section[4]

The physical properties, yields, and analyses are listed in Table I.

2-Carboxy-6,8-dichloro-γ-chromone (I).—A mixture of 11.8 g (0.080 mole) of ethyl oxalate and 15.0 g (0.075 mole) of 3,5-dichloro-2-hydroxyacetophenone[5] in 200 ml of anhydrous Et_2O was added, over a period of 30 min, to a vigorously stirred suspension of NaOEt (13.6 g, 0.2 mole) in 100 ml of anhydrous Et_2O. The mixture was kept at 20–25° for 0.5 hr, heated under reflux for 2 hr, cooled, and filtered. The sodium salt was suspended in 360 ml of a mixture of AcOH–concentrated HCl (5:1) and heated under reflux for 2 hr. The reaction mixture was cooled and the insoluble solid was collected. Recrystallization from AcOH gave 10.5 g (56%) of white needles.

Esters. General Method.—Concentrated H_2SO_4 (2 ml) was added slowly to a suspension of I (2 g) in 20 ml of the appropriate alcohol. The mixture was refluxed for 4 hr. The ester, which precipitated on cooling, was collected and washed ($NaHCO_3$, H_2O).

6,8-Dichloro-γ-chromone-2-carbonyl Chloride (VII).—The acid I was suspended in a mixture of 5 g of $SOCl_2$ and 6.0 ml of 1,2-dichloroethane and heated with occasional shaking under reflux for 7–8 hr. The hot mixture was filtered and the residue was extracted twice with hot petroleum ether (60–80°). The filtrate and the ethereal extracts were pooled and evaporated *in vacuo*. The residue was recrystallized from petroleum ether; yield 240 mg (6%) of pale yellow prisms.

2-(N,N-Diethylcarbonamide)-6,8-dichloro-γ-chromone (VI).—Diethylamine (0.5 ml, excess) was added to a cold suspension of 140 mg of VII in 3 ml of anhydrous C_6H_6. The mixture was kept at room temperature for 30 min and then refluxed for 30 min. The solvent was removed, and the residue was treated with H_2O, filtered, and washed (H_2O). The solid, recrystallized from EtOH, gave 100 mg (64%) of white needles.

Acknowledgment.—The author wishes to thank Professor Dr. Armando Novelli for his many helpful suggestions. This work was carried out with a grant from the C. N. I. C. y T.

(4) All melting points were taken in capillaries and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(5) A. B. Sen and P. M. Bhargara, *J. Indian Chem. Soc.*, **26**, 366 (1949).

Experimentally Induced Phenylketonuria. III. Inhibitors of Phenylalanine Hydroxylase Related to Esculetin

JOSEPH I. DEGRAW, MICHAEL CORY, W. A. SKINNER, MYNA C. THEISEN, AND CHOZO MITOMA

Life Sciences Research, Stanford Research Institute, Menlo Park, California 94025

Received November 17, 1967

In the first paper of this series[1] we reported some observations concerning the *in vitro* inhibitory action of several *o*-dihydroxy compounds and various simple phenylalanine derivatives on the enzyme, phenylalanine hydroxylase. Esculetin (6,7-dihydroxycoumarin) and 4-fluorophenylalanine were found to be the most effective inhibitors, confirming the work of other investigators.[2,3] Phenylalanine-derived alkylating agents, designed to be irreversible inhibitors, were further explored without success as reported in a second communication.[4] The subject of this paper is a further investigation, both *in vitro* and *in vivo*, of hydroxylated coumarin compounds related to esculetin.

We began our structure–activity investigation by preparing various 3- and 4-substituted 6,7-dihydroxycoumarins. We found that, *in vitro*, the 4-methyl, 4-*n*-butyl, and 4-phenyl analogs were more potent inhibitors of phenylalanine hydroxylase than esculetin. The 4-ethyl-, *n*-propyl-, and isopropyl-substituted compounds were about as active as esculetin, while the activity was considerably diminished for the 3-methyl and 3,4-dimethyl analogs (Table I).

TABLE I

In Vitro INHIBITION OF RAT LIVER PHENYLALANINE HYDROXYLASE[a]

Substituted coumarin	% inhib	Ratio of substrate: inhibitor
6,7-Dihydroxy- (esculetin)	55	100:1
Esculin	16	1:1
4-Methyl-6,7-dihydroxy-	77	200:1
	34	1000:1
4-Ethyl-6,7-dihydroxy-	55	100:1
4-*n*-Propyl-6,7-dihydroxy-	58	100:1
4-Isopropyl-6,7-dihydroxy-	53	100:1
4-*n*-Butyl-6,7-dihydroxy-	64	200:1
4-Phenyl-6,7-dihydroxy-	54	200:1
3-Methyl-6,7-dihydroxy-	41	50:1
3,4-Dimethyl-6,7-dihydroxy-	54	50:1
5,6-Dihydroxy-	52	10:1
7,8-Dihydroxy-4-methyl-	44	10:1
6,7,8-Trihydroxy-4-methyl-	55	100:1
5,6,7-Trihydroxy-	50	5:1
5-Hydroxy-4-methyl-	16	1:1

[a] See ref 1 for biological procedures.

We also investigated the effects of varying the position and number of hydroxyl groups while retaining either hydrogen or methyl at the 4 position. Both 5,6-dihydroxycoumarin and 7,8-dihydroxy-4-methylcoumarin were poor inhibitors as was 5,6,7-trihydroxycoumarin. 6,7,8-Trihydroxy-4-methylcoumarin was as potent as esculetin, but considerably less than 4-methylesculetin. 5-Hydroxy-4-methylcoumarin was a very poor inhibitor.

Two of the more active compounds, namely, 4-methyl- and 4-phenylesculetin, were selected for *in vivo* studies (Table II). Since esculin (the 6-glycoside

TABLE II

In Vivo INHIBITION OF RAT LIVER PHENYLALANINE HYDROXYLASE[a]

Compd	% inhib	Time of sacrifice after oral administration, hr
Esculin	82	0.5–1
4-Methylesculetin	70	3
4-Phenylesculetin	68	5

[a] Three 42–50-g Sprague–Dawley rats were used for each compound. Compounds were given orally as aqueous suspensions (pH 8–9) at 2 mmoles/kg. Animals were sacrificed at various intervals. The specific activity of the control liver phenylalanine hydroxylase in several experiments ranged from 0.113 to 0.193 μmole of tyrosine formed/hr per mg of protein.

(1) J. I. DeGraw, M. Cory, W. A. Skinner, M. C. Theisen, and C. Mitoma, *J. Med. Chem.*, **10**, 64 (1967).

(2) S. B. Ross and O. Haljasmaa, *Life Sci.*, **3**, 579 (1964).

(3) D. D. Watt and J. P. Vandervoorde, *Fed. Proc.*, **23**, 146 (1964).

(4) J. I. DeGraw, M. Cory, W. A. Skinner, M. C. Theisen, and C. Mitoma, *J. Med. Chem.*, **11**, 225 (1968).