THE PREPARATION OF THE OPTICAL ISOMERS OF α-(2 : 4-DICHLOROPHENOXY)PROPIONIC ACID AND THEIR GROWTH-REGULATING ACTIVITY

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α-(2 : 4-Dichlorophenoxy)propionic acid has been resolved into its optical isomers *via* the brucine and cinchonine salts, and the activity of the two forms, in three standard auxin tests, has been measured. It was found that the (+)-form had twice the activity of the racemic acid whereas the (—)-form was inactive.

Recent reports by Thimann (1951) on the activities of racemic and (+)-α-(2 : 4-dichlorophenoxy)propionic acids and by Wain (1951) & Åberg (1951) on the activities of (—)- and (+)-α-naphthoxypropionic acids have prompted us to report on some work carried out, mainly in 1948, on the preparation of (—)- and (+)-α-(2 : 4-dichlorophenoxy)propionic acids and on the results obtained with the isomers in some standard auxin tests.

Pure α-(2 : 4-dichlorophenoxy)propionic acid (23·51 g., m.p. 117°) was dissolved in 60 ml. of acetone by shaking. To the solution 39·44 g. of anhydrous brucine was added and dissolved by warming. The solution was stood in a refrigerator for a long period until a deposit of crystals was obtained. The crop was filtered, washed with a little acetone, and dried *in vacuo* over sulphuric acid. Crystals (30·45 g.) $[\alpha]^{17}_{5461}$ — 15·0° (*c*, 2·3 in acetone) was obtained. An approximate determination of the rotation of the brucine salt remaining in solution gave the value $[\alpha]^{17}_{5461}$ — 42°, hence the less soluble isomer would yield the (+)-isomer of the acid. The solid was repeatedly crystallized from acetone until the specific rotation became constant at $[\alpha]^{17}_{5461}$ — 11° (*c*, 1·33 in acetone), the melting point being 100°.

To isolate the (+)-acid, 14·6 g. of the purified brucine salt was dissolved in the minimum volume of warm alcohol and the solution was then stirred with a mixture of 150 ml. of water and 10 ml. of hydrochloric acid. The oil that formed soon changed to crystals, which were powdered and filtered after standing overnight. Crystallization from benzene gave 3·4 g. of colourless needles, melting point 124°, $[\alpha]^{21}_{D}$ + 26·6° (*c*, 1·23 in ethanol). No racemization of an aqueous alcoholic solution of the acid was observed over a period of ten weeks.

Pure α-(2 : 4-dichlorophenoxy)propionic acid (11·75 g.) and 14·72 g. of cinchonine were shaken with 60 ml. of methanol until they dissolved. Water (85 ml.) was added and a crystalline salt was obtained after standing a few days. The crystals were washed with a little dilute methanol and dried *in vacuo* over sulphuric acid. The dry crystals, 13·2 g., had $[\alpha]^{19}_{D}$ + 113° (*c*, 2·0 in methanol) and the cinchonine salt remaining in solution was found to be more strongly dextrorotatory, indicating that the less soluble salt would yield the (—)-isomer of the acid. After repeated crystallizations from aqueous methanol a salt having a constant specific rotation $[\alpha]^{21}_{D}$ + 110° (*c*, 2·0 in methanol) and of indefinite melting point was obtained.

To isolate the (—)-acid 3·5 g. of the purified cinchonine salt was dissolved in 25 ml. of methanol and added to 200 ml. of stirred 1% sulphuric acid. When the oil formed had crystallized, it was collected, washed with water and dried. The dry solid, 1·38 g., was crystallized from 5 ml. of benzene, giving 0·9 g. of colourless needles, melting point 124°, $[\alpha]^{20}_{D}$ — 26·3° (*c*, 1·22 in ethanol).

The absolute configuration of the two stereoisomers may be deduced by assuming that the one that is physiologically active corresponds to the physiologically active isomers of α-(1-naphthoxy)propionic and α-(2-naphthoxy)propionic acids. It has been shown that (+)-α-bromopropionic acid is a D-compound (Brewster, Hughes, Ingold & Rao, 1950) and Fredga & Matell (1951) have shown that (—)-α-bromopropionic acid gives rise by inversion to (—)-α-(1-naphthoxy)propionic acid and (+)-α-(2-naphthoxy)propionic acid on reaction with the appropriate sodium naphtholate. Both these compounds are active and both are D-compounds, therefore (+)-α-(2 : 4-dichlorophenoxy)propionic acid, which is shown below to be active, is presumably the D-compound.

Avena *curvature test* (Went & Thimann, 1937*a*).—In this test the isomers were assayed at three concentrations, 0·1, 1·0 and 10·0 p.p.m., each inserted value being the mean curvature of 8 to 10 coleoptiles (Table I).

The curvatures produced were very small and were much nearer the top of the decapitated coleoptile than with the natural auxin, indole-3-acetic acid ; this is indicative of a more limited facility for movement in the tissues. The most effective concentration was 1·0 p.p.m., which compares with an optimal concentration of 0·05–0·08 p.p.m. of indole-3-acetic acid. The (+)-isomer was active, the (—)- inactive ; the racemic compound gave an intermediate effect.

Table I

Isomer	(+)-			Racemic			(−)-		
Test	1	2	Total	1	2	Total	1	2	Total
Concn.									
0·1 p.p.m.	0·0	0·1	0·1	0·0	0·1	0·1	0·0	0·1	0·1
1·0 p.p.m.	5·5	2·4	7·9	2·1	1·0	3·1	0·0	0·0	0·0
10·0 p.p.m.	4·4	1·8	6·2	2·1	1·4	3·5	0·0	0·1	0·1
Totals	9·9	4·3	14·2	4·2	2·5	6·7	0·0	0·2	0·2

Tomato-parthenocarpy test (Luckwill, 1948).—This test method depends on the ability of the substance to stimulate parthenocarpic development of unpollinated tomato-ovaries. The growth response of the ovaries is expressed as a percentage of the optimum response under the conditions of the experiments and the probit response is then plotted against the logarithm of the dose. From the probit lines the median effective dose (ED-50) can be read off. In these tests each isomer was tested at 7 dosage rates, varying from 0·0225 to 2·25 μg. of acid per ovary, with 10 replicate ovaries for each dosage rate arranged in 5 randomized blocks to minimize the effect of temperature gradients in the glasshouse (Table II).

Table II

Test	Isomer	ED-50 μg. per ovary	Ratio of optically active to racemic
1	(+)-	0·22	2·13
	Racemic	0·47	1
2	(+)-	0·50	2·00
	Racemic	1·00	1
3	(−)-	No activity	0
4	(−)-	No activity	0

The results show that the (+)-isomer has approximately twice the activity of the racemic acid, whereas the (—)-isomer is without activity. It was found that the activity of the aqueous alcoholic solution of the (+)-isomer decreased with time. After 10 weeks it was 1·5 times as active; after one year it was only 1·2 times as active as the racemic acid, but it was not confirmed whether this was due to spontaneous racemization, although this appeared to be so.

Went pea test (Went & Thimann, 1937*b*).—In this test the curvatures of the split pea-stems were tested at five concentrations and the results given are each the mean of three determinations. Activity is assessed 0–6; 0 represents no activity and 6 the highest activity possible in the test (Table III).

Table III

Isomer	(Concn. tested, p.p.m.)				
	0·1	0·5	1	5	20
(+)-	5	6	6	6	6
Racemic	4	6	6	6	6
(−)-	0	0	0	0	1
Water control	0				

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