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STUDIES ON ARGENTINA PLANTS—XIX

ALKALOIDS FROM *CARDUUS ACANTHOIDES* L. STRUCTURE OF ACANTHOINE AND ACANTHOIDINE AND SYNTHESIS OF RACEMIC ACANTHOIDINE

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Abstract—*C. acanthoides* L. contains several alkaloids which can be separated into weak and strong bases. From the fraction of strong bases, two crystalline hydrochlorides could be isolated and the corresponding alkaloids named acanthoine (I) and acanthoidine (VI). The structure of acanthoine dihydrochloride, which has hypotensive activity in dogs, has been confirmed by the synthesis of the racemic isomer.

Carduus acanthoides L. is one of the several plants popularly named thistles, in Argentina. It was introduced from Europe into several South American countries. It adapts and grows with facility in temperate climates and in Argentina it is found in the provinces of Buenos Aires, Cordoba and Santa Fe, where it is considered a pest. The plants employed in our work were collected in the surroundings of the city of Buenos Aires.

The presence of alkaloids in *C. acanthoides* L. has been mentioned by Wall *et al.*[1] Dr. E. Hug,[2] while working with extracts of the plant many years ago, found that a crude fraction could be separated, with hypotensive activity in dogs, which gave a definite positive test for alkaloids. A more detailed examination of the hypotensive action was made by Barán[3] in Hug's laboratory.

In view of the connection of the hypotensive activity with a fraction rich in alkaloids, it was considered of interest to isolate the substances responsible for the activity.

The amount of bases present in *C. acanthoides* L. is very small and diluted extracts give only a very faint reaction with the usual alkaloidal reagents. On concentration, a clear positive reaction is obtained and paper chromatography reveals the presence of several bases.

After some preliminary trials, it was found that extraction of the dried stalks with methanol, at room temperature, gave a suitable preparation for the study of the bases present in the plant.

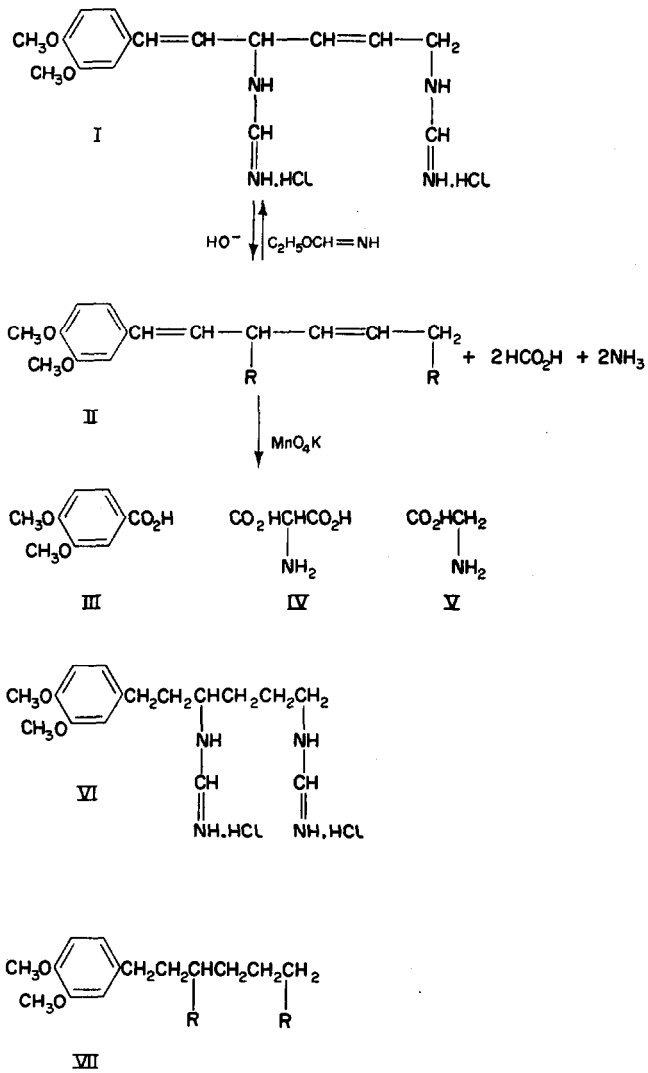
Fractionation of the methanolic extracts revealed the presence of two kinds of bases: weak bases, which in aqueous solutions at pH 7, could be extracted with chloroform, and stronger ones, which need pH 11 for their extraction. The crude

[1] M. E. Wall, C. S. Fenske, J. W. Garvin, J. J. Williams, Q, Jones, B. G. Schubert and H. S. Gentry, J. *Amer. Pharm. Assoc.* **48**, 695 (1959).

[2] Private communication

[3] L. de Pirro de Barán, Thesis, Facultad de Ciencias Médicas, Buenos Aires (1959).

mixture of the bases extracted at pH 7 was found devoid of interesting physiological activity and it was reserved for further study.



The hypotensive activity of the extracts was found in the crude fraction containing the strong bases. Paper chromatography revealed the presence of three alkaloids. Chromatography of the fraction, on alumina of pH 4·7, allowed the separation of the base of greatest mobility on paper (R_f 0·44), which could be crystallized as a dextrorotary dihydrochloride of formula $C_{16}H_{22}N_4O_2 \cdot 2HCl$. It was named acanthoine and was not physiologically active.

The remaining two bases (R_f 0·28 and 0·11) could only be separated by chromatography on a column of cellulose. The base with R_f 0·11 was also isolated as a dextrorotary dihydrochloride, of formula $C_{16}H_{26}N_4O_2 \cdot 2HCl$ and it had a clear hypotensive

activity. It was named acanthoidine. The medium moving base, which was devoid of hypotensive action, was not investigated.

The formation of a dihydrochloride indicated that only two of the nitrogen atoms of acanthoine (I) were basic and the same results were obtained by titration.

When acanthoine dihydrochloride was hydrolyzed with alkali it produced two moles of ammonia, two of formic acid and an optically active base $C_{14}H_{20}N_2O_2$ (II, R = NH_2), which was isolated as a crystalline dihydrochloride (II, R = NH_2·HCl). This base contained the two original methoxy groups of acanthoine and gave a N-diacetyl derivative (II, R = NH·$COCH_3$), which could be used for a molecular weight determination and confirmed the former formula.

The fact that the products of hydrolysis contained all the carbon and nitrogen atoms of the original alkaloid, that one mole of formic acid and one mole of ammonia were produced for each basic nitrogen remaining in II (R = NH_2), and that only two nitrogens were basic in acanthoine, suggested that two formimidino groups were present in it.

These groups should be responsible for the two bands at 1655 and 1682 cm^{-1}, which were found in the infrared spectrum of acanthoine dihydrochloride and which correspond to similai bands found in aromatic amidines by Fabian *et al.*[4]

Proof of the presence of the formamidino groups was obtained when the base (II, R = NH_2) on treatment with ethyl formimino ether, employing the method of Pinner, as used by Hill and Rabinowitz[5] for the preparation of amidines, regenerated acanthoine dihydrochloride (I). This was confirmed when it was found that this dihydrochloride when treated with *o*-phenylendiamine dihydrochloride produced benzimidazol, a reaction characteristic of the formamidines.

Oxidation of the base $C_{14}H_{20}N_2O_2$ with potassium permanganate, produced veratric acid (III), aminomalonic acid (IV) and glycine (V). The three compounds together contain all the original carbon and nitrogen atoms of the above base and indicate that its structure is that of 6-(3,4-dimethoxyphenyl)-1,3-diamino-hexa-2,5-diene (II, R = NH_2).

The structure of acanthoidine dihydrochloride was deduced easily when it was found that it could be obtained by catalytic hydrogenation of acanthoine dihydrochloride. Two moles of hydrogen were added with some difficulty and afterwards, at a slower rate, hydrogen was continuously absorbed. By stopping the hydrogenation at a convenient time, acanthoidine dihydrochloride could be isolated without difficulty.

Acanthoidine dihydrochloride is then represented by VI and it also gives on alkaline hydrolysis two moles of formic acid and two moles of ammonia and a saturated optically active base, $C_{14}H_{24}N_2O_2$. The latter was isolated as the dihydrochloride (VII, R = NH_2·HCl) and gave a crystalline N,N′-dibenzoyl derivative (VII, R = NHCOPh). This base is 1-(3,4-dimethoxyphenyl)-3,6-diamino-hexane (VII, R = NH_2) and its dihydrochloride was also obtained by hydrogenation of the dihydrochloride of the dienic base (II, R = NH_2·HCl) produced in the alkaline hydrolysis of acanthoine dihydrochloride. Treatment of VII (R = NH_2) with ethyl formimido ether regenerated acanthoidine dihydrochloride (VI).

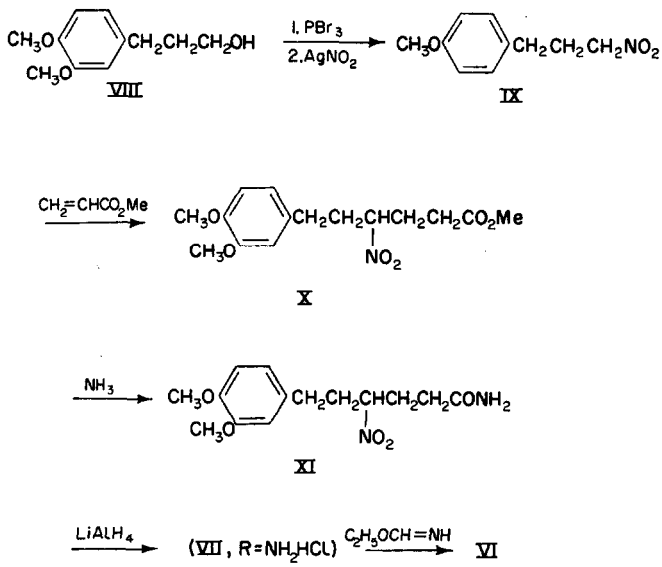
The structure of acanthoidine dihydrochloride was confirmed by synthesis of the racemic compound. 3,4-Dimethoxyphenyl-propanol (VIII), prepared by reduction

[4] J. Fabian, V. Delaroff and M. Legrand, *Bull. Soc. Chim. Fr.* 287 (1956)

[5] A. J. Hill and I. Rabinowitz, *J. Amer. Chem. Soc.* **48**, 732 (1926).

of 3,4-dimethoxyphenyl-propionic acid with lithium aluminium hydride, was transformed into the bromo compound which, treated with silver nitrate, yielded the expected 1-(3,4-dimethoxyphenyl)-3-nitropropane (IX).

This compound, submitted to a Michael reaction with methyl acrylate, gave the nitro ester (X), which was transformed into the amide (XI). Treatment of the amide with lithium aluminium hydride resulted in simultaneous reduction of the nitro and the amido groups, and (±)-1-(3,4-dimethoxyphenyl)-3,6-diamino-hexane was



isolated as dihydrochloride (VII, R = NH_2·HCl). The diabenzoyl derivative (VII, R = NH·COPh) gave an infrared spectrum identical with that obtained from the dextrorotary isomer prepared by benzoylation of VII (R = NH_2).

Treatment of the racemic dibase with ethyl formimidate produced racemic acanthoidine dihydrochloride (VI), which in chloroform solution gave an infrared spectrum identical with that of the optically active hydrochloride.

What is peculiar in acanthoine and acanthoidine is that they contain a formamidino group. There are a number of natural organic substances which contain the structure —NH—C=N forming part of a guanidino group or of a ring system. Natural compounds with a formamidino structure are scarce and have been found among short lived metabolic products, like formiminoglutamic acid,[6] formiminoglycine[7] or 5-formiminotetrahydrofolic acid.[8]

EXPERIMENTAL

Mp are uncorrected. UV spectra were always determined in ethanol 96%. Descending paper chromatography was employed, with Whatman paper No. 1, and *n*-butanol saturated with buffer solution of pH 5·6, as mobile phase. The buffer was prepared by mixing 90·5 ml 0·2M sodium acetate

[6] B. Borek and H. Neelsch, *J. Amer. Chem. Soc.* **75**, 1772 (1953); *J. Biol. Chem.* **205**, 459 (1953).
[7] J. B. Rabinowitz and N. E. Pricer, J. *Amer. Chem. Soc.* **78**, 1513 (1956).
[8] H. Tabor and L. Wyngarden, *J. Biol. Chem.* **234**, 1830 (1960).

and 9·5 ml 0·2M acetic acid. The reagent described by Munier and Macheboeuf[9] was used for development of the alkaloidal spots.

Preparation of the crude alkaloid extract. Clean stalks (500 kg) of *C. acanthoides* L. were dried at room temp, in diffuse light. The dried material was ground to a coarse powder weighing 47 kg. To 38 kg of this powder 150 l. of methanol were added, the mixture stirred at room temp for 24 hr and filtered. The residue extracted twice with 100 l. methanol under the same conditions. Evaporation of the methanolic extracts under vacuum, at 30°, yielded a sirupy residue with a strong alkaloidal reaction.

Ten liters of 10% acetic acid were then added to the residue, stirred for 10 hr and allowed to stand at 5° for 24 hr, when a green insoluble product separated, adhering to the walls of the container. Filter Cel was added in the necessary amount to obtain a filterable solid, 500 g of Darco incorporated, the whole mixture stirred for 1 hr and filtered. The remaining cake was extracted twice with 5 l. 10% acetic acid. The cake was discarded after the third extraction, although it gave a weak alkaloidal reaction.

The acetic extracts were united and carried to pH 7 by the addition of 50% NaOH with very good stirring and avoiding any increase of temp. It was then extracted ten times with 2 l. of chloroform. The chloroform extracts contained 2 bases (R_f 0·26 and 0·35), which were not further studied.

The aqueous phase was then alkalinized to pH 11 and extracted again with chloroform, until it gave a negative test with Mayer's reagent. Usually 20 extractions with 5 l. of chloroform were needed. The combined chloroform extracts were concentrated to 5 l., well dried and evaporated to dryness in vacuum. An oily residue, weighing 40 g, was obtained. On paper chromatography, three spots with R_f 0·11, 0·28, and 0·44 were observed.

Separation of the bases extracted at pH 11. A typical separation was carried out by dissolving 20 g of the residue in the minimum amount of chloroform and chromatographing it through a column containing 800 g of acid alumina (Woelm, grade III).

For the elution, chloroform with increased amounts of methanol was employed. Fractions of 500 ml were collected. The fractions obtained by elution with 1·5 and 2% methanol revealed on paper chromatography to contain only *acanthoine* (R_f 0·44). On evaporation they yielded a crude crystalline light brown mass from which crystalline *acanthoine dihydrochloride* could be prepared. 1·26 g of the crude compound were obtained from the 38 kg of dried powdered plant.

The fraction eluted with 5% to 20% methanol contained 2 bases (R_f 0·11 and 0·28). On evaporation an oily residue was obtained weighing 1·55 g. These two bases could only be separated by chromatography through a cellulose column.

Separation of the bases R_f 0.11 and 0·28. A column containing 50 g cellulose powder (Whatman) was prepared and equilibrated with n-butanol saturated with buffer of pH 5·6 which was also used as mobile phase. 500–600 mg of the residue, obtained by evaporation of the fraction eluted from alumina were dissolved in the same buffer mixture and run through the column. Fractions of 5 ml were collected. As an example, in a particular experiment, fractions 41–55 gave on paper chromatography only one spot (R_f 0·28), fractions 54–58 two spots, corresponding to the original bases, and fractions 59–73 only the spot R_f 0·11, which corresponds to *acanthoidine*. The last fractions were employed for the preparation of *acanthoidine dihydrochloride.*

(+)-*Acanthoine dihydrochloride* (I)

The yellowish crystalline solid (1·26 g; R_f 0·44) obtained from the chromatography on alumina was recrystallized from n-propanol and yielded 1·14 mg needles, m.p. 218–219°. After several recrystallizations from the same solvent, 1·05 g of crystals were collected, m.p. 221°; $(\alpha)_D^{29°}$ +7·1 ± 1·2° (*c*, 0·4, H_2O). (Found: C, 49·7; H, 6·2; N, 7·6 (titration), 14·5 (Dumas); Cl, 19·0; OCH_3, 16·4. $C_{16}H_{22}N_4O_2 \cdot 2HCl$, $0{\cdot}5H_2O$ requires: C, 50·0; H, 6·5; N, 14·6; Cl, 18·5; $2OCH_3$, 16·1%).

When the recrystallization was carried out from ethanol, with addition of ether to turbidity, needles m.p. 192–193° were obtained on several occasions. $(\alpha)_D^{29°}$ +7·0 ± 1·4°; λ max 238 m (log ε 4·5); 312 (3·3). (Found: C, 48·2, H, 6·1; N, 14·4; Cl. 17·6; OCH_3, 15·9. $C_{16}H_{22}N_4O_2 \cdot 2HCl$, H_2O requires: C, 48·8; H, 6·6; N, 14·2; Cl, 18·1; $2OCH_3$, 15·6%). Acanthoine dihydrochloride is hygroscopic and on standing decomposes with formation of coloured products. It is very soluble in water, methanol, ethanol and acetone. It can be dissolved in large amounts of chloroform; it is rather insoluble in ether and benzene.

[9] R. Munier and M. Macheboeuf, *Bull. Soc. Chim. Biol.* **38**, 846 (1951).

(+)-*Acanthoidine dihydrochloride* (VI)

All fractions containing only acanthoidine (R_f 0·11) from the cellulose chromatography of the original 1·55 g oily residue were united, diluted with 750 ml of water and freeze-dried. A solid residue (170 mg) was obtained, which crystallized from 96% ethanol, giving fine needles, m.p. 249–251°, $(\alpha)_D^{29°}$ +6·5 ± 1·5°. After several recrystallizations from ethanol, 140 mg were obtained. M.p. 250–251°. $(\alpha)_D^{29°}$ +6·8 ± 1·5° (*c*, 0·4, H_2O) λ max 230 mμ (log ε 4·5); 284 (4·2). (Found: C, 49·8; H, 7·1; N, 14·7; Cl, 18·3. $C_{16}H_{26}N_4O_2 \cdot 2HCl$ requires: C, 50·5; H, 7·1; N, 14·8; Cl, 18·4%).

Acanthoidine dihydrochloride is hygroscopic. It can be kept without decomposition. It is less soluble than acanthoine dihydrochloride in the usual solvents.

Alkaline hydrolysis of acanthoine dihydrochloride

Acanthoine dihydrochloride (100 mg) was dissolved in 7 ml of 10% NaOH, and boiled for 1 hr, while nitrogen was passed through the solution and bubbled through 2N HCl, to trap all volatile bases. During the operation, a brown oil separated in the flask containing the alkaloid. The ammonia retained in the hydrochloric acid solution was precipitated as ammonia sodium cobalto-nitrite and determined by the method of Wagner *et al.*[10] The alkaline solution was extracted with ether until the aqueous phase gave a negative Mayer test. The united ether extracts were washed with water, and the water added to the mother liquors remaining after the extraction with ether. This aqueous solution contained formic acid, which was determined following the indications of Grant[11]. (Found: 1·98 mole HN_3; 1·83 mole HCO_2H. $C_{16}H_{22}N_4O_2 \cdot 2HCl$ requires: $2NH_3$; $2HCO_2H$).

(+)-6-(3,4-*Dimethoxyphenyl*)-1,3-*diaminohexa*-2,5-*diene dihydrochloride* (II, R = $NH_2 \cdot HCl$)

The ether extracts from the alkaline hydrolysis of acanthoine dihydrochloride were evaporated to dryness yielding an oily brown residue. It was dissolved in n-propanol, acidified to pH 2 with conc HCl and kept overnight at 5° whereupon the dihydrochloride of a base crystallized as white needles. After drying it weighed 50 mg m.p. 220–222°. After recrystallization from ethanol, the m.p. was 227°; $(\alpha)_D^{29°}$ +30·9° ± 2·0 (*c*, 0·4; H_2O); λ max 236 mμ (log ε 9·3); 312 (3·1); R_f 0·24 (Found: C, 50·8; H, 6·6; Cl, 20·3, $C_{14}H_{20}N_2O_2 \cdot 2HCl$ requires: C, 51·3; H, 6·9; Cl, 21·1%). This dihydrochloride was used as a source of free base in all experiments described below.

A crystalline *dipricate* was obtained by testing the free base, obtained from the purified dihydrochloride, with the calculated amount of picric acid in ethanol. Recrystallized from ethanol it gave yellow prisms, m.p. 206–208°. (Found: N, 15·6; $C_{14}H_{20}N_2O_2 \cdot 2C_6H_3N_3O_7$ requires: N, 15·9%).

(+)-*Acanthoine dihydrochloride from* (+)-6-(3,4-*dimethoxyphenyl*)-1,2-*diaminohexa*-2,5-*diene*

To a solution of 50 mg of the base in 10 ml of absolute ethyl ether, 10 ml of ether, containing the formimino ether prepared from 100 mg ethyl formimino ether hydrochloride, were added and the mixture left for 10 days at room temp. The ether solution was then evaporated to dryness, the oily residue dissolved in 1 ml ethanol, acidified to pH 2 with conc hydrochloric acid and left at 0° for several hr, whereupon needles crystallized m.p. 218–220°. After recrystallization from n-propanol the product m.p. 221°; $(\alpha)_D^{29°}$ +7·0 ± 1·4° and had no depression when mixed with acanthoine dihydrochloride of m.p. 221°; λ max 238 mμ (log ε 4·5); 312 (4·5); R_f 0·44; all in agreement with the data obtained with the acanthoine dihydrochloride prepared from the plant.

Benzimidazol from acanthoine dihydrochloride

Acanthoine dihydrochloride (100 mg) was well mixed with 100 mg of *o*-phenylendiamine dihydrochloride and heated in a close tube at about 200° for 3 hr. After opening the tube, 1 ml water was added, the insoluble material filtered and the filtrate made alkaline by adding solid sodium hydrogen carbonate. The solution was kept at 0° overnight, whereupon white needles crystallized, m.p. 167–169° (50 mg). Recrystallized several times from water, the needles m.p. 170–171° and did not depress the m.p. of pure benzimidazol, m.p. 170–171°. U.V. and IR spectra were identical.

(+)-*N,N'-Diacetyl*-1,3-*diamino*-6-(3,4-*dimethoxyphenyl*)-*hexa*-2,5-*diene* (II R = $NHCOCH_3$)

The base obtained from 100 mg of the hydrochloride was dissolved in 2 ml acetic anhydride, 0·5

[10] C. D. Wagner, R. H. Brown and E. D. Peters, *J. Amer. Chem. Soc.* **69**, 2611 (1947).
[11] W. M. Grant, *Analyt. Chem.* **19**, 206 (1947).

ml pyridine added and the mixture boiled for 20 min. After cooling, 10 ml water were added and the solution freeze-dried. The residue was dissolved in a small amount of benzene, chromatographed through 2 g alumina (Woelm, activity III) and eluted with benzene, collecting fractions of 3 ml. In a typical experiment, fractions 3–7 yielded on evaporation a crystalline residue. The crystals were pooled and recrystallized from cyclohexane. Prisms m.p. 153–154°; $(\alpha)_D^{29°}$ +28·7 ± 2·0 (*c*, 0·4; ethanol). (Found: C, 65·5; H, 6·9; N, 8·4; M.w. (Rast) 325, 335. $C_{18}H_{24}N_2O_4$ requires: C, 65·1; H, 7·3; N, 8·4%; M.w. 332).

Oxidation of (+)-1,2-*diamino*-6-(3,4-*dimethoxyphenyl*)-*hexa*-2,5-*diene*

(a) *Veratric acid* (III). The base obtained from 500 mg of (+)-1,2-diamino-6-(3,4-dimethoxyphenyl)-hexa-2,5-diene dihydrochloride, was dissolved in 20 ml water, 5 ml of saturated sodium carbonate solution added and the mixture heated to 80°. Potassium permanganate (5% solution) was added slowly, in portions of 1 ml, until it was not further reduced. After elimination of the excess by passing sulfur dioxide, the acidic solution was extracted in a continuous apparatus with ethyl ether for 48 hr; the ether extracts dried and evaporated to dryness. The crystalline residue was recrystallized from water several times and had a m.p. 186–187°. It was identified as veratric acid (186–187°) by mixed m.p. and IR spectrum.

(b) *Glycine* (V). The aqueous acidic mother liquors remaining after the extraction of veratric acid were concentrated to dryness and the solid residue was extracted with 100 ml boiling ethanol, the insoluble material being discarded. Evaporation of the ethanolic solution yielded a residue which dissolved in 10 ml water. Elimination of most of the inorganic material present in this solution was accomplished by passing it through a column of Amberlite IR-120 in the acidic stage, washing with water, eluting with ammonia and collecting the ninhydrine positive eluates. These fractions were passed through another column of carbonate Amberlite IRA-410, which was first washed with water and then eluted with H NCl, recovering again all ninhydrine positive fractions. This solution was treated with silver carbonate, the insoluble material filtered and the remaining silver ions eliminated with hydrogen sulfide. Evaporation of the solution gave an oily residue which contained two ninhydrine positive substances. They were separated by chromatography on a column of Whatman cellulose (12 × 1·5 cm) equilibrated with a mixture of acetic acid: n-butanol: water (6:25:25) employing the same solvent for elution and collecting fractions of 5 ml. Fractions 6–18 and 22–31 gave a ninhydride positive reaction. They were diluted with water and freeze-dried. The residue obtained from fractions 22–31 gave in several systems a R_f identical with that of glycine. A small amount was transformed by the method of Sanger[12] into 2,4-dinitrophenyl-glycine. Chromatography, employing n-butanol saturated with water, gave R_f 0·35; with benzene saturated with acetic acid 1%, R_f 0.06, identical with those of an authentic sample.

The residue (20 mg) were dissolved in 1 ml water; 0·2 ml benzoyl chloride added and 1 ml 20% sodium hydroxide added slowly, with agitation. After 20 min conc HCl was added and the precipitate formed was filtered, dried and well washed with warm carbon tetrachloride. The insoluble residue was recrystallized several times from water. Prisms m.p. 186–187°, which gave no depression when mixed with pure huppuric acid of m.p. 188–189°.

(c) *Acetyl-aminomalonic acid* (VI). Freeze drying of fractions 6–18 gave 29 mg solid. This solid (100 mg) collected from several preparations, were dissolved in 10 ml 5% HCl solution, N NaOH added to faint turbidity and a clear solution obtained by the addition of the exact amount of diluent hydrochloride acid. This solution was cooled by the addition of some ice flakes, 1 ml acetic anhydride added and the mixture strongly shaken for 15 min. Sodium acetate (1 g) dissolved in 5 ml water was then added and on standing at 0° overnight, crystals appeared. They were collected and recrystallized from water–acetone, prisms, m.p. 133°.

Acetylamino-malonic acid was prepared for comparison by acetylation of a pure sample of amino malonic acid prepared by an identical procedure, m.p. 133°. (Found: C, 37·6; H, 4·6; N, 9·1. $C_5H_7NO_5$ requires: C, 37·3; H, 4·4; N, 8·69%). A mixture of both preparations gave the same m.p.

(+)-*Acanthoidine dihydrochloride by hydrogenation of* (+)-*acanthoine dihydrochloride*

To a solution of 500 mg of acanthoine hydrochloride in 50 ml ethanol, 2·5 ml acetic acid and 300 mg platimum oxide were added and the mixture was then hydrogenated for 7 hr at 3 atm. Paper chromatography showed that acanthoine was almost completely transformed into acanthoidine.

[12] F. Sanger, *Biochem. J.* **39**, 507 (1945); **40**, 261 (1946).

After filtering the catalyst, the solution was evaporated to dryness in vacuum at room temp, the oily residue dissolved in 5 ml n-propanol and conc hydrochloric acid carefully added to pH 2. By cooling, white needles crystallized, which were collected after standing overnight at 5°, m.p. 249–251° (380 mg). After recrystallization from 96% ethanol the m.p. 250–251° was obtained; $(\alpha)_D^{29}$ +6·7° ± 1·20°. The product was identical with acanthoidine dihydrochloride (mixed m.p., UV spectrum and paper chromatography).

(+)-1,3-*Diamino*-6-(3,4-*dimethoxyphenyl*)-*hexane dihydrochloride* (VII. R = NH_2·HCl)

Alkaline hydrolysis of (+)-*acanthoine hydrochloride.* Acanthoidine dihydrochloride (100 mg) were submitted to alkaline hydrolysis in the same way as described for acanthoine dihydrochloride. The ammonia and formic acid produced were determined. (Found: 1·97 mole NH_3 1·92 mole HCO_2H. $C_{16}H_{26}N_4O_2$·2HCl requires: $2NH_3$; $2HCO_2H$).

The ethereal extracts containing the basic moiety were evaporated, the brownish oily residue dissolved in 1 ml absolute ethanol, the solution acidified to pH 2 with conc hydrochloric acid, and ethyl ether added to faint turbidity. On standing overnight at 5°, white needles crystallized (45 mg), m.p. 219–220°. Recrystallized from absolute ethanol-ether, they melted 220–221°; $(\alpha)_D^{29°}$ ±25·4° ± 2·0° (*c*, 0·4; H_2O). λ max 230 mμ (log ε 5·7); 284 (5·3). (Found C, 51·7; H, 8·0; N, 8·6; Cl, 21·7 $C_{14}H_{24}N_2O_2$·2HCl requires: C, 51·7; H, 8·6; N, 8·6; Cl, 21·8%).

The same dihydrochloride was obtained when 200 mg of (+)-(3,4-dimethoxy-phenyl)-1,3-diamino-hexa-2,5-diene dihydrochloride, obtained from the alkaline hydrolysis of acanthoine dihydrochloride were hydrogenated in absolute ethanol at 3 atm press, using platinum oxide as catalyst, m.p. 220–221°; $(\alpha)_D^{29°}$ +25·2° ± 2·0°. When mixed with and authentic sample no depression in m.p. was observed. The UV spectrum and R_f values were identical.

(+)-*Acanthoidine dihydrochloride from* (+)-1,3-*diamino*-6-(3,4-*dimethoxyphenyl*)-*hexane*

(+)-1,3-Diamino-6-(3,4-dimethoxyphenyl)-hexane (30 mg) was dissolved in 10 ml absolute ether and treated with ethyl formimidate, exactly as described for the dibase derived from acanthoine. The residue obtained after working the reaction mixture was dissolved in 1 ml absolute ethanol, acidified to pH 2 with hydrochloride acid and left at 0°. Crystals precipitated with m.p. 247–249°, which when crystallized from 96% ethanol gave needles with m.p. 249–251°; $(\alpha)_D^{29°}$ +6·6 ± 1·1°. λ max 230 mμ (log ε 4·5); 284 (4·17); R_f 0·11. All data in agreement with those from acanthoidine dihydrochloride. The mixed m.p. of the natural and the regenerated dihydrochloride was not depressed.

(+)-*N,N'-Dibenzoyl*-1,2-*diamino*-6-(3,4-*dimethoxyphenyl*)-*hexane* (VII, R = NH·COPh)

(+) 1,3-Diamino-6-(3,4-dimethoxyphenyl)-hexane (50 mg) prepared from VII (R = NH_2·HCl), were treated with 0·5 ml pyridine and 0·15 ml benzoyl chloride in 1 ml benzene. The mixture was heated to 70° for 30 min, poured into 10 ml iced water and the suspension extracted with benzene. The benzene extracts, after washing with 5% sodium carbonate solution and water, were dried and evaporated to dryness in vacuum. The oily residue crystallized from ethanol 70% and was recrystallized from the same solvent in prisms, m.p. 127–128°; $(\alpha)_D^{29°}$ +21·3 ± 2° (*c*, 0·38, ethanol) λ max 284 mμ (log ε 5·2). (Found: C, 72·2; H, 6·9; N, 6·2. $C_{28}H_{32}H_2O_4$ requires: C, 72·1; N, 6·9; N 6·1%).

3-(3,4-*Dimethoxyphenyl*)-1-*propanol* (VIII)

3-(3,4-Dimethoxyphenyl)-propionic acid (14 g) were dissolved in 300 ml absolute ether and the solution added slowly to a well stirred suspension of 5 g lithium aluminium hydride in 200 ml of the same solvent. The temp increased and the ether boiled. Boiling was continued for 3 hr when 200 ml 20% sulfuric acid were added and a clear solution obtained. The lower aqueous phase was separated and extracted 3 times with 400 ml portions of ether. The ether extracts and the original ethereal phase were joined, washed with a small amount of water and evaporated, yielding an oily residue. 4 N NaOH (40 ml) were added and the resulting mixture extracted 3 times with 200 ml ether. Evaporation of the collected ether extracts gave an oil that was distilled in vacuum. The fraction boiling at 142–145° was (0·5 mm) separated and redistilled again, and the fraction boiling 142–134° (0·5 mm) was collected. A colorless oil (8 g) was obtained with a low solubility in water, soluble in the usual organic solvents. (Found: C, 67·8; H, 8·4; $C_{11}H_{16}O_3$ requires: C, 67·2; H, 8·2%).

The aqueous phase, remaining after the extraction with ether, was acidified and 3 g of crude (3,4-dimethoxyphenyl)-propionic acid were recovered.

3-(3,4-*Dimethoxyphenyl*)-1-*propyl*-3,5-*dinitrobenzoate*

This was prepared by mixing 1 ml of the alcohol with 2 ml pyridine, adding 1 g of 3,5-dinitrobenzoyl chloride, heating to dissolution, allowing the mixture to cool to room temp and then pouring it into 10 ml ice-water. The precipitate formed was recrystallized several times from ethanol; yellow needles, m.p. 106–107°. (Found: C, 55·7; H, 3·9; N, 7·1. $C_{18}H_{18}N_2O_7$ requires: C, 55·4; H, 4·6; N, 7·2%).

3-(3,4-*Dimethoxyphenyl*)-1-*nitropropane* (IX)

3-(3,4-Dimethoxyphenyl)-1-propanol (8 g) were dissolved in 20 ml carbon tetrachloride, the solution cooled at 3–5° and 8 g phosphorus tribromide added, maintaining the low temp. The mixture was then allowed to reach room temp and boiled for 45 min. Carbon tetrachloride (20 ml) were added, the solution washed with 5% sodium carbonate, then with water, dried and evaporated in vacuum. A yellow oil remained, which was distilled and a colorless fraction boiling 128° (4 mm) collected. The 3-(3,4-dimethoxyphenyl)-1-bromopropane was used without further purification. It was dissolved in 50 ml absolute ether and 4 g solid silver nitrite, finely ground with 4 g quartz added. The mixture was shaken for 2 days at room temp. The insoluble material was then filtered and the solution evaporated, leaving an oil which was distilled in vacuum, the fraction b.p. 155–160° (2 mm) separated and distilled again, collecting the fraction b.p. 158° (2 mm). A light yellow oil (2·2 g) was obtained. It was insoluble in water, soluble in NaOH with orange color, soluble in the usual organic solvents. (Found: C, 58·7; H, 6·7; N, 6·0. $C_{11}H_{15}NO_4$ requires: C, 58·7; H, 6·7; N, 6·2%).

(±) *Methyl*-6-(3,4-*dimethoxyphenyl*)-3-*nitrohexanoate* (X)

Methyl acrylate (300 mg) were mixed with 2·2 g 3-(3,4-dimethoxyphenyl)-nitropropane, 0·18 g triethylamine added and the solution left for 6 days at 37°. The mixture was then distilled, the fraction with b.p. 130–132° (5 mm) collected and redistilled again, collecting the fraction b.p. 130–131° (0·5 mm). It was a light yellow oil (500 mg) insoluble in water, soluble in N NaOH with an orange yellow color, soluble in the usual organic solvents. (Found: C, 57·8; H, 6·8; N, 4·5. $C_{15}H_{11}NO_6$ requires: C, 57·9; H, 6·8; N, 4·8%).

(±)-6-(3,4-*Dimethoxyphenyl*)-3-*nitrohexanamide* (XI)

The former methyl ester (1 g) was added to 20 ml methanol saturated with ammonia and the solution kept at 5° for 48 hr. It was then evaporated to dryness in vacuum and the solid residue recrystallized several times from ethanol (70%). Prisms, m.p. 38–39° were obtained (200 mg). With a low solubility in water and ethyl ether, they were soluble in methanol, ethanol, benzene and chloroform. (Found: C, 56·6; H, 6·8; N, 9·4. $C_{14}H_{20}N_2O_5$ requires: C, 56·8; H, 6·8; N, 9·5%).

(±)-1,3-*Diamino*-6-(3,4-*dimethoxyphenyl*)-*hexane dihydrochloride* (VII, R = NH_2·ClH)

(±)-6-(3,4-Dimethoxyphenyl)-3-nitrohexanamide (200 mg) were dissolved in 20 ml tetrahydrofurane and the solution slowly added to a suspension of 1 g lithium aluminium hydride in 20 ml of the same solvent. The mixture was then boiled for 7 hr and the excess of lithium aluminium hydride decomposed in the usual way.

The solution was made alkaline and well extracted with ether. The other extracts after washing with water and drying were evaporated, yielding 100 mg of a white oil, which on paper chromatography have an R_f 0·09, identical to that given by the base obtained from the alkaline hydrolysis of acanthoidine dihydrochloride. The oil was dissolved in 2 ml dried ether and gaseous hydrogen chloride passed through the solution. A precipitate appeared which was filtered and recrystallized from absolute ethanol–water, giving prisms m.p. 210–211° λ max 230 mμ (log ε 5·7) 284 (5·3). (Found: C, 72·9; H, 7·1; N, 6·1. $C_{28}H_{32}N_2O_4$ requires: C, 73·0; H, 7·0; N, 6·1%).

(±)-N,N′-*Dibenzoyl*-1,3-*diamino*-6-(3,4-*dimethoxyphenyl*)-*hexane* (VII) R = NHCOPh)

This product was prepared by treatment of the base from the racemic dihydrochloride with a mixture of benzoyl chloride–pyridine, as was described for the natural base. On recrystallization from ethanol, prisms m.p. 134–135° were obtained. λ max 284 mμ (log ε 5·17). UV (in ethanol) and IR spectra in chloroform were identical to those from the dibenzoyl derivative of the natural optically active base (m.p. 127–128°).

(±) *Acanthoidine dihydrochloride* (VII)

(±)-1,3-Diamino-6-(3,4-dimethoxyphenyl)-hexane (30 mg) obtained from the dihydrochloride were dissolved in 10 ml dry ether, treated at room temp with 10 ml of an ethereal solution containing the ethyl formimidate from 100 mg ethyl formimidate hydrochloride and left for 10 days. The solution was then evaporated to dryness, the residue dissolved in 0·5 ml ethanol and conc hydrochloric acid added to pH 2. By cooling for several hours at 0°, crystals of (±) acanthoidine hydrochloride precipitated, which after recrystallization from ethanol m.p. 234–235° (needles). (Found: C, 72·9; H, 7·1; N, 6·1. $C_{28}H_{32}N_2O_4$ requires: C, 73·0; H, 7·1; N, 6·1%).

This salt in paper chromatography gave R_f 0·11. Its UV had λ max 230 mμ (log ε 4·5), 284 (4·2) in agreement with the R_f and UV spectrum of (+)-acanthoidine hydrochloride (with a m.p. 249–251°) prepared from *C. acanthoides*. IR spectra in chloroform were also identical.

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