[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

THE ERGOT ALKALOIDS. X.* ON ERGOTAMINE AND ERGOCLAVINE

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Ergotinine, for which the empirical formula $C_{35}H_{39}N_5O_5$ was derived by Barger and Carr,[1] has been shown by us[2] to be composed of lysergic acid, ammonia, isobutyrylformic acid, *d*-proline, and *l*-phenylalanine, joined in amide linkages, a fact in agreement with such a formulation. Similarly, the simpler ergot alkaloid, ergometrine, now accepted as possessing the formula $C_{19}H_{23}N_3O_2$,[3] we have shown to yield lysergic acid and 2-aminopropanol-1 on hydrolysis, and is therefore the hydroxyisopropylamide of lysergic acid.[4] Preliminary notes have also been presented on the results of similar hydrolytic studies with ergotamine[5] and ergoclavine.[6] Our examination, from this standpoint, of these alkaloids as well as that of ergotoxine has since been carried farther.

The ergotamine employed in these studies was purchased as the tartrate from the Sandoz Chemical Works. The free base was separated from the salt and was then submitted to alkaline hydrolysis as described in the experimental part. The formula for ergotamine (and ergotaminine) has been well established by its discoverer, Stoll,[7] to be $C_{33}H_{35}N_5O_5$. This formula differs from that of ergotinine by C_2H_4. Since in our first experiments no evidence of the formation of isobutyrylformic acid from ergotamine on alkaline hydrolysis could be obtained, it appeared possible that pyruvic acid, which differs from the first acid by C_2H_4, might be in question. Unfortunately, the instability of pyruvic acid under the conditions of

* For paper IX of this series see *J. Biol. Chem.*, **113**, 767 (1936).

[1] G. BARGER AND F. H. CARR, *J. Chem. Soc.*, **91**, 337 (1907).

[2] W. A. JACOBS AND L. C. CRAIG, *J. Biol. Chem.*, **110**, 521 (1935).

[3] A. STOLL AND E. BURCKHARDT, *Compt. rend.*, **200**, 1680 (1935); W. A. JACOBS AND L. C. CRAIG, *Science*, **82**, 16 (1935); H. W. DUDLEY, *J. Am. Chem. Soc.*, **57**, 2009 (1935).

[4] W. A. JACOBS AND L. C. CRAIG, *Science*, **82**, 16 (1935).

[5] W. A. JACOBS AND L. C. CRAIG, *ibid.*, **81**, 256 (1935).

[6] W. A. JACOBS AND L. C. CRAIG, *J. Am. Chem. Soc.*, **57**, 960 (1935).

[7] *Swiss Patents*, No. 79879 (1918) and No. 86321 (1919). A. STOLL, *Schweiz. Apoth. Ztg.*, Nos. 26–28, pp. 134, 136–138 (1922).

hydrolysis makes its detection more difficult than that of isobutyrylformic acid in the case of ergotinine. If ergotamine is heated for a short time in alkaline solution the resulting mixture gives a red color with nitroprusside similar to that given by pyruvic acid, and a color which changes after addition of ammonium chloride through purple to blue. Such a reaction is not given by ergotinine where isobutyrylformic acid is in question. This experience with ergotamine was confirmed by the preparation of a phenylhydrazone, although in extremely poor yield, which proved to be identical with that of pyruvic acid. This was prepared by acidification and extraction of the mixture obtained after short alkaline hydrolysis. During the latter, partial evolution of ammonia was readily detected. On resumption of the alkaline hydrolysis, the cleavage was completed and from the reaction mixture lysergic acid was readily obtained.

Paralleling our earlier experience with ergotinine, an appreciable amorphous, amphoteric fraction was recovered which was further hydrolyzed by hydrochloric acid. Phenylalanine (almost entirely racemized) was readily isolated from this hydrolysate after removal of Cl ions. The mother liquor from phenylalanine was desiccated and the residue was esterified with methyl alcohol and hydrochloric acid. A basic amino acid ester fraction was obtained in the usual manner, which on fractionation in a micro still gave a distillate at the proper temperature and pressure for proline methyl ester. This was identified by conversion into the double gold salt $C_6H_{11}NO_2 \cdot HAuCl_4$. Thus, ergotamine and therefore its isomer ergotaminine consist of lysergic acid, ammonia, proline, phenylalanine, and pyruvic acid combined in amide linkage.

In order to determine the optical character of the amino acids present in ergotamine, the latter was then hydrolyzed directly with hydrochloric acid. After removal of the deeply colored products arising from the decomposition of lysergic acid, it was a comparatively simple matter to obtain phenylalanine which, as in the case of ergotinine, proved to be *l*-phenylalanine, although partly racemized ($[\alpha]_D^{25} = -23°$). From the filtrate of the latter, the methyl ester was prepared as usual and the fraction corresponding to proline methyl ester was obtained in the micro still. Unfortunately, the amount was insufficient to redistill for analysis. However, its rotation, $[\alpha]_D^{25} = +20°$, indicated, as in the case of ergotinine, that we were again dealing with the unnatural form of an amino acid, *i.e.*, *d*-proline.

The occurrence of the unnatural form of an amino acid is so unique that at one stage of our studies we sought for a possible explanation of its formation in the process of isomerization of the alkaloids, such as ergotoxine into ergotinine and ergotamine into ergotaminine. The possibility was considered that such isomerization might involve enolization with participation of the hydrogen atom on the asymmetric α-carbon atom of proline, with

racemization of the latter. And since other asymmetric centers are still present elsewhere in the large molecule, the resulting substances would be epimers and not true enantiomorphs. Hence, ergotinine could be one of these easily crystallizing epimers and so yield *d*-proline on hydrolysis. If this theory were valid, ergotoxine should then yield *l*-proline. Ergotoxine (the ethane sulfonate of Burroughs Wellcome and Company), however, on acid hydrolysis also yielded *d*-proline methyl ester of $[\alpha]_D^{25} = +34°$ (c = 0.570). Therefore, such an explanation of the occurrence of *d*-proline in these alkaloids must be discarded. Although this observation is of interest in confirming our experiences with ergotinine and ergotamine in regard to the optical character of the proline constituent, we now know that another explanation is necessary for the isomerization noted in the cases of the pairs of alkaloids. As we are presenting elsewhere, the center of such isomerization is to be found in lysergic acid itself and the evidence at hand indicates that the shift of the double bond of the latter is responsible.

We have similarly attempted the study of ergoclavine which was first described by Küssner.[8] Our experience with this alkaloid has convinced us that it is a difficult matter to obtain it in homogeneous form. When prepared by the method given by Küssner, it was readily isolated from the crude alkaloid mixture and agreed in general properties with those described by him. In Küssner's report a formulation for the alkaloid of $C_{31}H_{37}N_5O_5 \cdot H_2O$ was derived, or $C_{31}H_{37}N_5O_5$ for the anhydrous substance (dried at 80° and 1–2 mm.). Our experience has shown that such a formulation requires revision. Our analytical data with the anhydrous substance, as given in the experimental part, are in excellent agreement with the figures of Küssner. However, the results of our hydrolytic studies indicate that the formulation of ergoclavine should be more properly $C_{25}H_{30}N_4O_4$. When ergoclavine was hydrolyzed by alkali, lysergic acid was obtained by the usual procedure, and its identity was confirmed by the preparation of the methyl ester. Ammonia was also collected as the hydrochloride during the hydrolysis. When the attempt was made to isolate isobutyrylformic acid by the method used in the case of ergotinine, only a few milligrams of a phenylhydrazone of doubtful homogeneity could be obtained. The conclusion appears warranted that pure ergoclavine does not contain this component. The statement in our preliminary note, based on results obtained with an impure alkaloid, that it contains isobutyrylformic acid is therefore withdrawn. When ergoclavine is hydrolyzed for a short time with alkali, it is possible to obtain a positive red color test for pyruvic acid with nitroprusside, which changes on addition of ammonium chloride through purple to blue. However, we were not able to isolate a

[8] W. KÜSSNER, *E. Merck's Jahresbericht*, **47**, 5 (1933).

pure phenylhydrazone of pyruvic acid. The very small amount of phenylhydrazone which was obtained as stated above proved to be nonhomogeneous, and there is a suggestion that it consisted of a mixture of the phenylhydrazones of pyruvic acid and isobutyrylformic acid. The latter probably has its origin in a small amount of ergotinine which contaminates the ergoclavine and which was difficult to remove by repeated recrystallization. This was also suggested by the investigation of the amino acid fraction.

When the amphoteric fraction which remained after separation of lysergic acid was further hydrolyzed by acid, an amino acid was readily isolated, and gave analytical figures required by leucine. It was optically inactive. No phenylalanine could be obtained, and, although the leucine mother liquor after concentration gave an appreciable pyrrole reaction, it was not possible to isolate either proline or hydroxyproline. The pyrrole test, we believe, was probably caused by the presence of a small amount of proline having its origin in a small amount of contaminating ergotinine. Acid hydrolysis confirmed the results of the alkaline hydrolysis. Lysergic acid was destroyed as usual by the process, and after removal of chloride ions an amino acid was readily obtained, which from the analysis and rotation proved to be *l*-leucine. Its optical activity indicated partial racemization; $[\alpha]_D^{25} = -7°$ ($c = 1.0$ in water), which changed to $[\alpha]_D^{25} = +10°$ on acidification with hydrochloric acid. This change of the rotation sign excludes isoleucine. No phenylalanine and no proline could be isolated from the material which remained in the leucine mother liquors.

If it is accepted that ergoclavine consists of lysergic acid, ammonia, *l*-leucine, and pyruvic acid in amide linkages, its formula should be $C_{25}H_{30}N_4O_4$. Our analytical figures obtained with the alkaloid, as well as those reported by Küssner, are in excellent agreement with the requirements of such a formulation.

On repeated recrystallization from alcohol not only the analytical figures but the optical activity of ergoclavine appeared to remain constant. Nevertheless, such apparent constancy of the rotation does not entirely exclude the possibility that it may be a mixture of isomers. According to Kreitmair,[9] ergoclavine possesses a specific biological action quantitatively indistinguishable from that of ergotoxine. However, contrary to the other active ergot alkaloids which are levorotatory in chloroform solution, ergoclavine is strongly dextrorotatory. We have observed for the air-dry alkaloid $+104°$ ($c = 0.5$ in chloroform), and Küssner reported $+115°$ ($c = 0.5$ in chloroform). This might suggest a mixture of isomeric ergoclavines of the ergotinine and ergotoxine types. Another possibility also comes to

[9] H. KREITMAIR, *ibid.*, **47**, 13 (1933).

mind: namely, that ergoclavine may belong in a third isomeric group (ψ ergotinine of Smith and Timmis?[10]). This point, however, will be discussed at greater length elsewhere in connection with the discussion of isomeric lysergic acids and derivatives. The above formulation for ergoclavine must be advanced, however, with necessary reservations until at least the work can be repeated with unquestionably homogeneous material.

EXPERIMENTAL

Alkaline hydrolysis of ergotamine.—A solution of 0.6 g. of ergotamine tartrate (Sandoz Chemical Works) was treated with excess sodium carbonate solution, and extracted with warm chloroform. After drying of the extract, the chloroform was removed *in vacuo*. The residue was dissolved in methyl alcohol and the solution was again concentrated to remove any chloroform. The amorphous residue was treated with a solution containing 3 cc. of water, 3 cc. of methyl alcohol, and 0.18 g. of potassium hydroxide. The solution was refluxed in a current of hydrogen for 15 minutes during which ammonia was evolved. Water (3 cc.) was then added to the mixture, and methyl alcohol was quickly removed under reduced pressure. The remaining solution, about 5 cc. in volume, was cooled in ice and treated with dilute sulfuric acid until acid to Congo red. It was then extracted with 10 cc. of ether. The aqueous layer was further treated as described below.

The clear ether extract was carefully concentrated to a small oily residue which was treated with a few milligrams of phenylhydrazine. The mass partially crystallized. It was taken up in a very small volume of dilute acetic acid and the crystals were collected. The crystalline material was combined with the same fraction from a previous 0.3-g. run. The combined weight was 4.2 mg. It was recrystallized from a drop of glacial acetic acid; 2.6 mg. was thus obtained, which melted at 191° and was indistinguishable from the phenylhydrazone of synthetic pyruvic acid melting at 191.5°. A mixture melting point showed no depression.

Anal. Calc'd for $C_9H_{10}N_2O_2$. C, 60.68; H, 5.67.
Found: C, 61.27; H, 5.70.

The acid aqueous solution which remained after the above ether extraction was treated with excess sodium carbonate solution and then evaporated to dryness *in vacuo*. To the residue was added a solution containing 5 cc. of water, 5 cc. of methyl alcohol, and 1.4 g. of potassium hydroxide. The solution was refluxed in an atmosphere of hydrogen for 45 minutes. Water was added and the volume was then reduced to about 10 cc. *in vacuo* in order to remove the methyl alcohol. After cooling, the solution was made slightly acid to Congo red with sulfuric acid. The solid material was collected and the filtrate was set aside to be treated further as described below.

The solid material was extracted with ammoniacal methyl alcohol and the undissolved inorganic material was filtered off. The somewhat colored filtrate was evaporated to dryness. The residue was treated with 5 cc. of methyl alcohol, heated to boiling, and then cooled; 0.12 g. of crystalline lysergic acid was collected with methyl alcohol. Recrystallization from water gave 0.09 g. of substance crystallizing in leaves. It showed a decomposition point of about 228° depending on the rate of heating.

Anal. Calc'd for $C_{16}H_{16}N_2O_2$: C, 71.69; H, 6.00; N, 10.45.
Found: C, 71.56; H, 5.83; N, 10.48.

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[10] S. Smith and G. M. Timmis, *J. Chem. Soc.*, **1931**, 1888.

For further characterization, the lysergic acid was esterified by treating its suspension in dry acetone with excess diazomethane. The solid material soon dissolved. After evaporation to dryness, the residue was treated with ether. The ether solution was filtered from a small amount of amorphous material, and again concentrated. After recrystallization twice from benzene, it melted at 168°. The melting point of a mixture with the methyl ester of lysergic acid showed no depression.

The filtrate from the crude lysergic acid fraction was combined with a similar fraction from a previous 0.3-g. run. After treating with an excess of sodium carbonate, the solution was evaporated to dryness *in vacuo*. The residue was extracted with hot ethyl alcohol which removed most of the inorganic material. The extract was evaporated to dryness, and the residue was treated with 25 cc. of concentrated hydrochloric acid. After digesting on the steam bath overnight, the mixture was evaporated to dryness. The residue was dissolved in water and treated several times with boneblack in order to remove the dark-colored, amorphous material. The filtrate remained very dark-colored. It was treated with excess ammonia and then boiled down to a volume of about 2 cc. Upon cooling, crystallization occurred; 80 mg. of crystalline material was collected with a small volume of water. After boneblacking and recrystallization, colorless leaves were obtained which had all the properties of phenylalanine. The material decomposed at about 258° depending somewhat on the rate of heating.

Anal. Calc'd for $C_9H_{11}NO_2$: C, 65.45; H, 6.72; N, 8.48.
Found: C, 65.41; H, 6.60; N, 8.27.

The filtrate from the crude phenylalanine was evaporated to thorough dryness *in vacuo*. The residue was treated with 10 cc. of absolute methyl alcohol and saturated with dry hydrogen chloride. After standing at room temperature for 2 hours, the solution was evaporated to dryness. The residue was treated with several cc. of absolute methyl alcohol and evaporated to dryness again. The residue was dissolved in 2 cc. of methyl alcohol and treated with excess powdered potassium carbonate. The solid was filtered off and washed with a few drops of methyl alcohol. The filtrate was then treated with powdered calcium oxide and filtered. The filtrate was concentrated *in vacuo* to a syrup at a low temperature, and 10 cc. of anhydrous ether was added. The somewhat sticky, insoluble material was ground with a stirring rod in order to extract all the ether-soluble ester. The clear ether filtrate was concentrated to a syrup which weighed 100 mg. This was promptly fractionated in a micro still; 50 mg. of colorless oil distilled at the proper temperature for the methyl ester of proline, *i.e.*, 85–90° at 15 mm. A 15-mg. portion of the oil was dissolved with chilling in a few drops of 10 per cent. hydrochloric acid. A small volume of gold chloride was added and the crystals were collected with dilute hydrochloric acid. The product weighed 20 mg. and melted at 152°.

Anal. Calc'd for $C_6H_{11}NO_2 \cdot HAuCl_4$: C, 15.35; H, 2.57; Au, 42.04.
Found: C, 15.69; H, 2.67; Au, 42.14.

Acid hydrolysis of ergotamine.—The free amorphous alkaloid obtained, as in the previous case, from 1 g. of ergotamine tartrate was treated with 25 cc. of hydrochloric acid (sp. gr., 1.19) and digested on the steam bath overnight. The dark-colored solution was evaporated to dryness. The residue was treated with a small volume of water, and then with a saturated solution of silver sulfate until no more silver chloride was precipitated. The filtrate was saturated with hydrogen sulfide. After removal of excess hydrogen sulfide, the filtrate was carefully treated with barium hydroxide solution until all sulfate ion was just removed. The clear, somewhat colored filtrate was concentrated to a smaller volume and boneblacked. Not all

the color was removed by boneblack. The filtrate was again boiled down until crystals began to appear and the mixture was then chilled. The crystalline material was collected with a few drops of water, and the filtrate was set aside to be treated for the proline fraction as described below. The crystals weighed 80 mg. The material was dissolved in water and boneblacked. The colorless filtrate, on concentration, yielded colorless leaves which were collected with a few drops of water; 45 mg. of material was thus obtained, which decomposed at 260°.

$$[\alpha]_D^{25} = -23° \ (c = 0.6 \text{ in water})$$

Anal. Calc'd for $C_9H_{11}NO_2$: C, 65.41; H, 6.72.
Found: C, 65.47; H, 6.45.

The above filtrate from the crude, partly racemized phenylalanine was concentrated to dryness and esterified with methyl alcohol and hydrogen chloride exactly as described for the isolation of the methyl ester of proline under the alkaline hydrolysis. Upon fractionation, the fraction corresponding to the methyl ester of proline was too small to get satisfactory analytical figures, although a rotation was obtained; $[\alpha]_D^{25} = +20°$ ($c = 0.45$ in methyl alcohol).

Ergoclavine.—For the preparation of this alkaloid from the crude alkaloid mixture, the directions of Küssner[8] were essentially followed. The crude alkaloid was recrystallized from 90 per cent. alcohol. It formed microscopic platelets as described by Küssner. It began to soften at 175° and slowly melted at 176–177° to a resin which cleared only on heating a few degrees higher. (Küssner gives 170–171°.)

$$[\alpha]_D^{25} = +104° \ (c = 0.54 \text{ in chloroform for the air-dry substance})$$

For analysis the substance was dried at 120° and 15 mm.

Anal. Calc'd for $C_{25}H_{30}N_4O_4$: C, 66.63; H, 6.71; N, 12.44.
Found: C, 66.43; H, 6.57; N, 12.47.

After recrystallization from 90 per cent. alcohol.

$$[\alpha]_D^{25} = +104.5° \ (c = 0.535 \text{ in chloroform})$$

Anal. Found: C, 66.58; H, 6.70.

Other samples gave similar figures.

Küssner reported the following analyses for his substance which was dried at 20° and therefore still contained solvent (H_2O ?).

Anal. Calc'd for $C_{25}H_{30}N_4O_4 \cdot H_2O$: C, 64.07; H, 6.89; N, 11.97.
Found: C, 64.19, 64.30; H, 6.75, 6.77; N, 11.74, 12.16.

Küssner found that his substance lost 3.17 per cent. (apparently water) at 80° and 1–2 mm. Calculated for 1 mole H_2O, 3.84.

Alkaline hydrolysis of ergoclavine.—A 0.2-g. portion of ergoclavine was treated with 4 cc. of a solution containing 2 cc. of methyl alcohol, 2 cc. of water, and 0.56 g. of potassium hydroxide. It was refluxed in an atmosphere of hydrogen for 45 minutes. The methyl alcohol was removed under reduced pressure, and 2 cc. of water was added. The chilled mixture was made slightly acid to Congo red with sulfuric acid and extracted with 10 cc. of ether. The acid aqueous layer was treated for the lysergic acid fraction as described below. The ether solution was carefully concentrated to a syrup which was fractionated in a micro still. Approximately 2.5 mg. of colorless oil distilled. It was treated with an equivalent of phenylhydrazine. The crystalline product was recrystallized from glacial acetic acid. A small amount of crystals was thus obtained; melting point, 152–154°. This suggested the derivative of isobutyrylformic acid, but the amount obtained was too small for identifica-

tion and, at any rate, negligible when compared with the yield of this substance obtained under similar conditions from ergotinine.

The acid aqueous layer was filtered and the collected solid was treated with ammoniacal methyl alcohol. The undissolved inorganic salts were filtered off and the filtrate was evaporated to dryness. The residue was treated with 5 cc. of methyl alcohol and heated to boiling, leaving crystalline lysergic acid, which was collected, and recrystallized from water (50 mg. of broad leaves); decomposition on rapid heating at about 238°.

Anal. Calc'd for $C_{16}H_{16}N_2O_2$: C, 71.69; H, 6.00.
Found: C, 71.85; H, 6.02.

A portion of the above-mentioned acid was converted into the ester with diazomethane. It was recrystallized from benzene and melted at 168°. A mixture melting point with the ester of lysergic acid showed no depression.

$$[\alpha]_D^{25} = +86° \ (c = 0.3 \text{ in methyl alcohol})$$

During the above alkaline hydrolysis the hydrogen which bubbled through the boiling mixture was passed through dilute hydrochloric acid. This, on concentration, gave 15 mg. of ammonium chloride. Calculated, 23 mg.

The above-mentioned acid aqueous mother liquor from the crude lysergic acid and salts was treated with alcohol to remove salts, and filtered. The filtrate was concentrated to dryness and then hydrolyzed, as in the case of ergotamine, with concentrated hydrochloric acid. A dark-colored solution resulted which, after boneblacking and removal of chloride ions, gave a crystalline amino acid on concentration to a few cc., and addition of alcohol. Successive fractions were obtained and combined for recrystallization. The product melted above 290° and was optically inactive. Analysis showed it to be *d,l*-leucine.

Anal. Calc'd for $C_6H_{13}NO_2$: C, 54.93; H, 9.99.
Found: C, 55.58, 54.92; H, 10.16, 9.77.

Acid hydrolysis of ergoclavine.—Ergoclavine (0.8 g.) was treated with 25 cc. of concentrated hydrochloric acid (sp. gr., 1.19) and heated on the steam bath overnight. The acid solution was concentrated to dryness *in vacuo*. The residue was then taken up in water and filtered through boneblack. The deeply colored filtrate was treated with an excess of silver sulfate. The filtrate was saturated with hydrogen sulfide and after removal of excess reagent was filtered. The filtrate was carefully treated with the exact amount of barium hydroxide to remove all sulfate ions, and after filtration was concentrated to 10 cc. and boneblacked. The still somewhat colored solution was brought to about 2 cc. and treated with 2 cc. of ethyl alcohol. After cooling, 80 mg. of broad, thin leaves were collected. The mother liquor on further concentration yielded a second crop of crystals which weighed 10 mg. The combined fractions were recrystallized with boneblacking from 50 per cent. ethyl alcohol. The substance had all the properties of leucine. It melted at 297–300°.

Anal. Calc'd for $C_6H_{13}NO_2$: C, 54.93; H, 9.99.
Found: C, 55.04; H, 10.14.

The rotation showed it to be partially racemized *l*-leucine. $[\alpha]_D^{25} = -7°$ ($c = 1.0$ in water). Upon acidifying with hydrochloric acid, the rotation became $[\alpha]_D^{25} = +10°$. The mother liquor from leucine gave a positive pyrrole test, and the presence of proline was therefore suggested. However, an attempt to isolate it as the methyl ester, as described for the degradation of ergotamine, gave an oil which proved on distillation to be a mixture. The results obtained in the attempt to fractionate this

mixture were not of a conclusive, clear-cut nature. The total methyl ester fraction weighed 0.27 g.

To determine whether the pyrrole test might have had its origin in hydroxyproline, the residue obtained after extraction of the above-mentioned free methyl ester was examined for this amino acid by the method of Lang, as modified by Waldschmidt-Leitz and Akabori.[11] This proved to be definitely negative.

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[11] K. LANG, *Z. physiol. Chem.*, **219**, 148 (1933); E. WALDSCHMIDT-LEITZ AND S. AKABORI, *ibid.*, **224**, 187 (1934).