106. *The Selective Absorption of Optical Antipodes by Proteins.*

By William Bradley and Gerald C. Easty.

Both wool and casein selectively absorb (+)-mandelic acid from an aqueous solution of (±)-mandelic acid at room temperature. (+)-α-Naphthylglycollic acid is selectively absorbed by wool from an aqueous alcoholic solution of the (±)-acid.

The significance of these results is discussed.

The possibility that optical antipodes might be absorbed by proteins to different degrees appears to have been considered first by Willstätter (*Ber.*, 1904, **37**, 3758). He brought together wool and an aqueous solution of racemic tropacocaine, atropine, or homatropine but in no instance was the selective absorption of an optically active form observed. Ingersoll and Adams (*J. Amer. Chem. Soc.*, 1922, **44**, 2930) extended the study to weak acids. They prepared two azo-dyes by diazotising the (+)- and the (−)-form of α-*p*-aminobenzamido-α-phenylacetic acid and coupling the products with dimethylaniline. In preliminary experiments it was considered that the two dyes were absorbed by wool at different rates, but a more extensive investigation by Brode and Adams (*ibid.*, 1926, **48**, 2193, 2202) led to the conclusion that the rate of absorption of the two antipodes was the same. Earlier, Porter and Hirst (*ibid.*, 1919,

* Dr. E. Glueckauf (*Nature*, 1950, **166**, 775, and private communication) has recently developed a theory which accounts for the decrease in K_H^{Na} with increasing X_{NaR} and for the increase in selectivity with increase in the degree of cross-linking. According to this view, the predominant factor is not elastic forces of the network acting on the internal system but rather a combination of ion hydration and ion-pair formation between cations and resin anions. The degree of ion-pair formation is modified by the nature of the neighbouring cations, and is thus dependent on composition.

41, 1264) had referred to the partial resolution of a racemic dye by selective absorption on wool, and Porter and Ihrig (*ibid.*, 1923, **45**, 1990) showed that the optically active forms of the azo-compound obtained by diazotising (+)- and (−)-*m*-aminomandelic acid and coupling the products with β-naphthol combined with wool at different rates. Brode and Adams (*loc. cit.*) and Henderson and Rule (*J.*, 1939, 1568) were unable to confirm Porter and Ihrig's work. Morgan and Skinner (*J.*, 1925, 1731) showed that the (−)-form of 2 : 3-di-[*p*-(*p'*-sulphophenyl-azo)anilino]butane was less rapidly absorbed by wool than the (+)-form. Brode and Adams (*loc. cit.*) have pointed out, however, that the difference in the observed rates was within experimental error. Brode and Adams reached the conclusion that in no known case was one dye absorbed more rapidly than its mirror image or an active solution produced by contact of a racemic dye solution with wool or silk. In the meantime von Euler and Bucht (*Z. anorg. Chem.*, 1923, **126**, 269) showed that α-bromopropionic acid was absorbed by casein but that under the conditions employed selective absorption could not be observed. In a later investigation Brode and Adams (*J. Amer. Chem. Soc.*, 1941, **63**, 923) applied to wool and a synthetic fibre the (+)-, (−)-, and (±)-forms of the bisazo-compound derived by combining 2 : 2′-diamino-1 : 1′-dinaphthyl with phenyl-J-acid; there was no evidence of selective absorption. Recently, Kögl, Faber, and de Boer (*Rec. Trav. chim.*, 1950, **69**, 482) have prepared two stereoisomeric colouring matters by condensing tetra-aminophenazine with the (+)- and the (−)-form of camphorquinone; the two isomers showed no significant difference in absorption by animal tissues, either normal or malignant.

Brode and Adams (*loc. cit.*, 1941) remarked that the structure of the colouring matters used in tests for selective absorption might not have been the most suitable for the purpose. Kögl, Faber, and de Boer (*loc. cit.*) expressed a similar view in regard to their own experiments. It was considered that the asymmetric centres of the dyes were too far removed from their point of attachment to the tissues for asymmetry to have influenced the absorption process or the properties of the resulting complex. The difficulty of observing selective absorption is greater when the specific rotation of the test substance is low (von Euler and Bucht, *loc. cit.*). Particular interest attaches therefore to the work of Martin and Kuhn (*Z. Electrochem.*, 1941, **47**, 216) who investigated the absorption of mandelic acid on wool. Their experiments included a temperature effect, the wool being used in the form of a continuous strip which traversed in turn a cold and a hot aqueous solution of mandelic acid. The development of a lævorotatory solution was observed in several experiments but the results were variable. Regarding the degree of resolution achieved, the authors observe " Er ist so klein dass es schwierig sein würde ihn unmittelbar zu beobachten."

In the present investigation a study has been made of the absorption of mandelic acid on wool from an aqueous solution at room temperature. In preliminary experiments wool was first extracted by means of organic solvents and then brought into contact with dilute aqueous solutions of acetic and chloroacetic acids. In each instance a lævorotatory solution was obtained. This result indicates how doubtful may be the value of observations which consist solely in determining the rotation of solutions left in contact with wool. In the main experiments wool purified by solvent extraction was immersed in a cold aqueous solution of (±)-mandelic acid. The solution became lævorotatory and from it was isolated mandelic acid having in a typical experiment α_D −0·45°. The process of isolation consisted in recovering the solute, extracting it with ether, dissolving the ether-soluble portion in benzene, and adsorbing it from the benzene solution on a column of alumina. Washing with benzene containing 1—5% of acetone removed impurities, and then the mandelic acid was recovered from the column by extraction with dilute hydrochloric acid. Finally, it was extracted from the acid solution into ether. Separate experiments proved that none of the stages of purification brought about the resolution of (±)-mandelic acid; in fact a slight loss of activity was generally observed. Mandelic acid absorbed by the wool was then recovered by extraction with dilute aqueous ammonia or dilute hydrochloric acid and then taken into ether. The ether-soluble portion was transferred to benzene, then purified by adsorption on alumina, washing with benzene containing 1—5% of acetone, the mandelic acid being recovered as before by extraction with dilute hydrochloric acid and transference into ether. In a typical experiment mandelic acid recovered from wool had $[\alpha]_D$ +0·6°. The effect of ammonia in the process of isolating mandelic acid was studied in separate experiments. It was shown that dilute ammonia in contact with wool gave solutions that were always lævorotatory. The isolation of both lævorotatory and dextrorotatory mandelic acid in these experiments establishes the selective absorption of dextrorotatory mandelic acid from its aqueous solution by wool.

The experiments with wool have been extended to casein. A sample of casein purified by

means of organic solvents readily gave lævorotatory solutions when extracted in the cold with dilute acetic, chloroacetic, or benzoic acid or ammonia. In contact with solvent-extracted casein an aqueous solution of (±)-mandelic acid became lævorotatory and the process of purification adopted in the experiments using wool gave lævorotatory mandelic acid. In this instance the mere observation of lævorotation in the solution is valueless because of the high specific rotation of casein. A few milligrams of casein in solution cause lævorotation of the same magnitude as that due to the residual mandelic acid. Mandelic acid absorbed by the casein was extracted by means of dilute ammonia, and recovered and purified as described in the experiments using wool. It proved to be dextrorotatory with $[\alpha]_D$ +0·8°.

Experiments with a second sample of good quality commercial casein gave much smaller resolutions. After the casein had been powdered, however, resolutions were obtained which approached those afforded by the first sample. A third sample gave resolutions of the same order as the first. The amount of mandelic acid absorbed and the degree of resolution attained depended on the particle size. One sample of casein was divided into 30/40- and 150/200-mesh portions, each of which was treated as just described. The mandelic acid recovered from the aqueous solution in contact with the coarser casein had α_D −0·02°, that from the more finely divided casein mixture had α_D −0·10°. A second sample of casein, divided into 50/60- and <200-mesh portions, similarly gave unabsorbed acid having −0·02° and −0·06°, respectively. In each experiment the absorbed mandelic acid was also recovered; its dextrorotation was higher the more finely divided the casein had been.

The resolution of mandelic acid on wool and casein supports the view that the first result of the interaction of acids and proteins is the formation of a protein–acid salt, the process being analogous in principle to the resolution of acids by means of active alkaloids. No resolution could be detected when a synthetic polyamide (nylon) was used instead of wool or casein. Resolution on wool or casein does not rigidly prove the occurrence of salt formation with acids, as was once believed (*locc. cit.*; Willstätter; Ingersoll and Adams; Brode and Adams, 1924; Porter and Ihrig), for resolutions have been achieved on optically active neutral substrates. Tsuchida, Kobayashi, and Nakamura (*Bull. Chem. Soc. Japan*, 1936, **11**, 38) have shown that (±)-chlorobis(dimethylglyoxime)amminocobalt can be resolved on quartz, and Kayagunis and Coumolos (*Nature*, 1938, **142**, 162) have demonstrated the resolution of the chromium complex $Cr[(en)_3]Cl_3$ on the same substrate. Moreover, Henderson and Rule have resolved (±)-*p*-phenylenebisiminocamphor on activated lactose. In the present experiments it was found that mandelic acid in benzene–light petroleum could not be resolved on lactose or quartz, but the result may have been determined by very small adsorption.

In further experiments it has been shown that α-naphthylglycollic acid also is partly resolved when its solution in aqueous alcohol is brought into contact with wool. In one experiment the adsorbed acid had $[\alpha]_D$ +1·0°.

It is probable that the selective absorption of optical antipodes of resolvable acids shown by wool and casein will be shown also by many other proteins yet to be studied. In this connection the increase in resolving power of a given weight of a protein with decrease in particle size is of interest. It would suggest that the thin membranes of proteins which occur in natural structures may have in relation to their mass a very marked capacity for the selective absorption of optically active anions.

Experimental.

The optical rotations of the solutions referred to in the following experiments were measured at room temperature in a 2-dm. fixed-end-plate polarimeter tube containing 17·5 c.c. of solution, and values of α_D quoted are thus for $l = 2$. The end-plate error of the polarimeter tube was less than 0·005°. The source of illumination was a sodium-vapour lamp. Each optical reading was the mean of ten observations, the total maximum deviation never exceeding 0·01°. The polarimeter was a "Hilger" model, Type M 413. It afforded ample intensity of illumination with maximum sensitivity with the colourless or pale yellow solutions examined.

The mandelic acid was supplied by British Drug Houses, Ltd. Before use it was recrystallised twice from benzene. The m. p. was 118·5°.

The casein employed in most of the experiments was described as "calcium free" (B.D.H.). Before use it was extracted (Soxhlet) with absolute alcohol for 8 hours, and then with ether for the same period. Finally, the solvent was removed by heating the casein at 100° for 2 days. The purified casein was then kept in a vacuum-desiccator at 1—2 mm. pressure until required.

The wool was used in the form of long unwoven fibres or woven material. In either case the wool, generally about 50 g., was extracted with ether in a Soxhlet for 8 hours and then with absolute alcohol for the same period, to remove oils and grease. It was washed with five or six 3-l. portions of distilled water and then kept in 3 l. of very dilute acetic acid for 1 hour to remove traces of soluble material which was generally present in our samples of wool. The wool was finally washed with ten 3-l. portions of

distilled water and dried in a current of warm air for 6 hours. It was stored over calcium chloride in a desiccator until required.

Absorption of Mandelic Acid on Wool.—In a typical experiment 60 g. of purified wool fibres were immersed in 1 l. of a solution of 30 g. of (±)-mandelic acid in distilled water. The containing flask was stoppered and then kept at the room temperature for 10 days. The solution was separated by filtration, the wool washed with two 200-c.c. portions of distilled water and squeezed as dry as possible The filtrate and washings were combined and then concentrated to 35 c.c. The resulting solution was pale yellow, and had α_D −0·33°.

The total solution was evaporated to dryness, the residue dried in a desiccator over calcium chloride for 1 day, and then digested with dry ether and passed through a fine-mesh sintered-glass filter. A whitish residue gave a strongly positive result in the ninhydrin test. The filtrate was evaporated to dryness and the residue dissolved in absolute ethyl alcohol, to give 35 c.c. of pale yellow solution, having α_D −0·30°.

The ether-insoluble residue, dissolved in warm 3·5% hydrochloric acid (18 c.c.) and cooled, had α_D −0·07°.

The optically active alcoholic solution was evaporated to dryness and the residue recrystallised from toluene. The resulting crystals (m. p. 118·5°) were dissolved in ethyl alcohol to give 17·5 c.c. of a colourless solution, having α_D −0·02°.

The toluene mother-liquor was evaporated to dryness, and the residue dissolved in ethyl alcohol to give 17·5 c.c. of a solution having α_D −0·55°. The solution was then evaporated to dryness, the residue dissolved in cold benzene, and the solution passed through a column of alumina. Two pale brown bands formed, the one narrow and sharp, the other broad and diffuse. The bands were eluted with benzene containing 5% of acetone and then with acetone, and the combined eluates concentrated to 18 c.c.; the solution had α_D −0·02°.

The alumina column was extruded, digested with cold 3·5% hydrochloric acid, and the digest filtered. The fitrate was extracted three times with twice its volume of ether and the ethereal extracts were combined and evaporated to dryness. The residue, recrystallised from a small quantity of benzene, had m. p. 118°. The mandelic acid was dissolved in ethyl alcohol to give 17·5 c.c. of a solution, having α_D = −0·45°.

In later experiments it was found that the brown impurities could be removed from the mandelic acid by extracting its aqueous solution with approximately one-tenth of its volume of benzene. Only a small relative volume of benzene was used because the optically active forms of mandelic acid appeared to have a higher solubility in benzene than the racemic form. The small loss in activity resulting from this process of purification was similar to that when alumina was used.

The mandelic acid adsorbed on the wool was recovered by immersing the wool fibres in 1 l. of 0·1N-ammonia for an hour at the room temperature. The solution was filtered, evaporated to small volume, acidified, and then extracted three times with twice its volume of ether. The aqueous solution, concentrated to 18 c.c., had α_D = −0·02°.

The ethereal extract, which was pale yellow, was evaporated to dryness and the residue (6·5 g.) dissolved in ethyl alcohol to give 18 c.c. of a solution, having α_D +0·45°, corresponding to $[\alpha]_D$ +0·6°.

The alcoholic solution was evaporated to dryness and the bulk of the residue recrystallised from toluene. The crystals which separated were dissolved in ethyl alcohol to give 17·5 c.c. of solution, α_D +0·03°.

The toluene filtrate was evaporated to dryness and the mandelic acid thus recovered was dissolved in ethyl alcohol to give 17·5 c.c. of a solution, α_D +0·40°.

The alcoholic solution was evaporated to dryness and the residual mandelic acid dissolved in cold benzene. The benzene solution was passed through a column of alumina and the brown bands which formed were eluted. The mandelic acid retained on the alumina was recovered by extraction with 3·5% hydrochloric acid. The acid solution was extracted with ether, the ethereal solution evaporated, and the residue recrystallised from a small volume of benzene. The mandelic acid, m. p. 118°, which separated, was dissolved in ethyl alcohol to give 17·5 c.c. of a solution, α_D +0·35°.

The solution containing the brown impurities eluted by means of benzene and acetone was concentrated to 18 c.c., the solution then having α_D +0·02°.

Absorption of Mandelic Acid on Casein.—Casein (40 g.) was added to a solution containing (±)-mandelic acid (15 g.) in absolute alcohol (100 c.c.). After the mixture had been kept for 6 days at the room temperature in a stoppered flask, the casein was filtered off and the filtrate concentrated; its optical rotation was α_D −0·78°. The solution was evaporated, the residue (10·3 g.) extracted by means of ether, and the solute recovered. Its optical rotation in absolute alcohol was α_D −0·58°. The solute, crystallised from toluene, gave mandelic acid, α_D −0·08°. The toluene mother-liquor, chromatographed on alumina, afforded a second crop of mandelic acid, m. p. 118°, having α_D −0·41° in absolute alcohol.

The mandelic acid absorbed by the casein was extracted by immersion for an hour in 0·1N-ammonia. The solution was filtered, and the filtrate acidified, and then extracted by ether. Evaporation of the ethereal extract afforded 3·6 g. of a residue having α_D +0·35°, $[\alpha]_D$ +0·8°. A benzene solution of the residue, chromatographed on alumina, afforded mandelic acid, m. p. 118°, α_D +0·21°.

Action of Weak Acids and Ammonia on Wool.—In a series of experiments wool was left in contact with dilute aqueous solutions of acetic and chloroacetic acids. The solutions became lævorotatory and very little of the optical activity was transferred, when the solutions were extracted by means of ether.

Similar experiments were carried out with dilute aqueous ammonia, the filtered extracts being acidified before being extracted by ether. The results are shown in the table.

Reagent.	Amount.	Amount of wool, g.	Temp.	Time (hrs.).	Fraction of reagent examined.	Optical rotation.* (A.)	(B.)
0·2N-(±)-Mandelic acid	1 l.	60	15°	240	Not absorbed	−0·66°	−0·60°
					Absorbed	—	+0·45
0·2N-Acetic acid	1 l.	60	15	240	Not absorbed	−0·16	−0·01
0·2N-Chloroacetic acid	1 l.	60	15	240	Not absorbed	−0·31	−0·02
0·1N-Ammonia	200 c.c.	20	15	1	Not absorbed	−0·007	—
0·1N-Ammonia	200 c.c.	20	50—60	2	Not absorbed	−0·02	—

* $l = 2$.

(A) Aqueous extract as separated. (B) Aqueous extract extracted by means of ether, the ethereal extract evaporated, and the residue dissolved in water.

The experiment on the absorption of mandelic acid on casein was repeated with benzoic acid (12 g.) instead of (±)-mandelic acid (15 g.). α_D corresponding to (A) in the table was −0·18°, and corresponding (B) −0·02° (not absorbed) and −0·02° (absorbed) ($l = 2$).

Absorption of α-Naphthylglycollic Acid on Wool.—Wool fibres (30 g.) were immersed for 6 days at the room temperature in a solution containing (±)-α-naphthylglycollic acid (8 g.; m. p. 98—99°; prepared from chloral and α-naphthylmagnesium bromide by McKenzie and Dennler's method, *J.*, 1926, 1599), in a mixture of water (500 c.c.) and alcohol (300 c.c.). The unabsorbed acid was recovered (α_D −0·54°) and suspended in water, the suspension shaken with a small volume of benzene, and the undissolved acid recovered (α_D −0·53°). The absorbed acid (4·4 g.), recovered by means of ammonia and purified as for the unabsorbed acid, had α_D +0·50°, $[\alpha]_D$ +1·0°.

In an identical experiment using (±)-mandelic acid (6·0 g.), the unabsorbed acid had α_D −0·35°, and the absorbed acid α_D +0·31°.

In these experiments the rotations were measured in ethyl alcohol.

To estimate the extent of loss of optical activity in dissolution and recovery of mandelic acid, (−)-mandelic acid was submitted to the following process. The acid (5·02 g.), dissolved in ethyl alcohol to give 17·5 c.c. of solution (α_D −1·24°) was recovered by evaporation and dissolved in distilled water (200 c.c.), the solution kept at 90—95° for 6 hours, and the acid recovered by evaporation (5·00 g.) and dissolved in ethyl alcohol to give 17·5 c.c. of solution, which then had α_D −1·22°.

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