310. *The Selective Absorption of Optical Antipodes by Proteins. Part II.**

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The study of the selective absorption of (+)-mandelic acid on wool (*J.*, 1951, 499) has been extended to mandelic acid derivatives. The relation between the amounts of acid absorbed and resolved has been determined, and, in addition, the stability of the wool protein–mandelic acid complexes. These results, together with parallel observations on the stability of the salts of mandelic acid with L-arginine and L-lysine, suggest that the resolution of mandelic acid on wool occurs at the L-arginine and L-lysine residues, and mainly, if not entirely, in the crystalline regions of the fibre.

In a previous communication * it was shown that wool and casein selectively absorb (+)-mandelic acid from an aqueous solution of (±)-mandelic acid at room temperature. The resolution was attributed to the formation of salts by the union of wool-protein with the (+)- and the (−)-acid, that with the (+)-acid being the more abundant. The absorption of mandelic acid by wool has now been examined more closely, and it has been found that under conditions similar to those described in Part I equilibrium is reached after 5—6 days at 31·3°, and that three-quarters of the acid ultimately bound combines within 4 min. of contact. At equilibrium the excess of (+)- over (−)-acid combined amounts to 3·5%; after 4 min. it is 2·1%. Resolution of the acid thus occurs from the beginning of the absorption. After the first 4 min. the increase in the excess of (+)-acid combined is proportional to the increase in the total acid combined.

When brought into equilibrium with aqueous (±)-mandelic acid at pH 2·5 and 31·3°, 1 kg. of wool combines with 0·45 mole of acid. The result accords with that reported for hydrochloric acid (0·4 mole) and chloroacetic acid (0·44 mole) at 0° and pH 2·5 (Steinhardt, Fugitt, and Harris, *J. Res. Nat. Bur. Stand.*, 1940, **25**, 519; 1941, **26**, 293), and with the same authors' observation that the amount of hydrochloric acid combined is almost independent of temperature between 0° and 50°.

The absorption of mandelic acid by wool is not accompanied by hydrolysis of the wool-protein or by structural changes other than those which accompany salt-formation. Wool which had been brought into equilibrium with mandelic acid during 5 days, then freed of acid, again equilibrated with mandelic acid under the same conditions, and finally rendered

* The previous paper, with the same general title (*J.*, 1951, 499), is now regarded as Part I of the series.

acid-free, showed the same behaviour as the original wool when immersed in aqueous (±)-mandelic acid. Further, the total absorption and resolution of mandelic acid remained unaltered when the period of contact was increased from 5 to 15 days.

The protein–acid complex prepared by immersing wool in aqueous (±)-mandelic acid remained unchanged in composition when transferred to a second portion of the same solution. The complex prepared from wool and an aqueous solution of pure (−)-mandelic acid, however, changed in composition when transferred to an aqueous solution of the (±)-acid, some of the combined (−)-acid being replaced by the (+)-form. The rate of replacement was low, only 3% of the combined (−)-acid being replaced in 4 days at 15°.

It is generally considered that the acid-binding capacity of wool-protein is due mainly to the presence of arginine and lysine, and in lesser degree histidine, residues. For this reason we have investigated the union of L-arginine and L-lysine with mandelic acid. Each of these combines with mandelic acid and each forms a product containing more of the (+)- than of the (−)-acid. L-Arginine forms a white crystalline salt, $C_6H_{14}O_2N_4,2C_8H_8O_3$. When crystallised from a solution of the components in absolute alcohol, or in a mixture of equal volumes of alcohol and water, the salt contains the (+)- and the (−)-form of mandelic acid in the ratio 1·02 : 1·00. The same salt is formed when a solution of (±)-mandelic acid in alcohol is added to solid L-arginine. In these circumstances the yield of salt is smaller, the degree of resolution is higher (20—30% instead of 2—3%), and the occurrence of resolution coincides with the separation of the salt on the surface of the L-arginine.

The salt containing approximately 2% more of the (+)- than the (−)-acid remained unaltered in composition after 8 days' contact with an alcoholic solution of the (±)-acid at 15°. The salt prepared analogously from pure (−)-mandelic acid, however, altered in composition in contact with the (±)-acid, a portion of the (−)-acid being replaced by the (±)-form from the solution. A feature of the replacement reaction was the low rate at which it occurred; only 4% of the combined acid was replaced in 15 days. The result indicates that the high degrees of resolution observed when alcoholic (±)-mandelic acid is brought into contact with solid L-arginine arise from a difference in the rates of the initial reaction of (+)- and (−)-mandelic acid with L-arginine, and not from subsequent replacement of the (−)-acid by the (+)-form. The close similarity in the behaviour of wool and L-arginine towards (±)-mandelic acid is also evident.

The replacement of (−)- by (+)-mandelic acid in combination with wool-protein is interesting in relation to other phenomena. Elöd (*Trans. Faraday Soc.*, 1933, **29**, 327) found that wool immersed in aqueous hydrochloric acid containing the disodium salt of 1-α-naphthylazo-2-naphthol-3 : 6-disulphonic acid combined first with hydrogen and chloride ions, and that the latter were subsequently replaced by anions of the dye. The mandelic acid experiment shows that interchange of anions can take place on wool independently of a difference in composition between the anions.

It is generally held that wool consists of amorphous and crystalline forms of a protein, the amorphous regions being the more accessible. All parts of the fibre are ultimately reached by acids of small molecular dimensions (Astbury and Dawson, *J. Soc. Dyers and Col.*, 1938, **54**, 6).

The present results, together with parallel studies on the desorption of mandelic acid from wool, suggest the following sequence of events. First, mandelic acid is taken up rapidly by the readily accessible amorphous regions of wool-protein and then more slowly by the crystalline portions. The second phase is characterised by the constancy of the ratio, $\frac{\text{excess of (+)- over (−)-acid absorbed}}{\text{total acid absorbed}}$, which suggests that the composition of the wool-protein concerned is uniform.

There was no evidence of resolution when degummed silk was brought into contact with aqueous (±)-mandelic acid. The result could be due to the smaller proportion of basic amino-acids in silk protein, but another possibility is that resolution occurs most readily in the crystalline regions of proteins, and it is known that the more highly orientated regions of silk do not contain either L-lysine or L-arginine (Trogus and Hess, *Biochem. Z.*, 1933, **260**, 376; Coleman and Howitt, *Nature*, 1945, **155**, 78).

We have extended the experiments with mandelic acid to several of its substitution

products including *o*-, *m*-, and *p*-nitro-, *p*-methoxy-, and *o*-ethoxy-mandelic acid. Each of these was absorbed and resolved. In contrast *m*-(*p*-hydroxyphenylazo)mandelic acid was not resolved. In other experiments it has been shown that mandelic acid is resolved by L-lysine but not by L-histidine or L-glutamic acid.

Experimental

Combination of L-Arginine with (±)-Mandelic Acid.—(*a*) L-Arginine (0·992 g., $[\alpha]_D$ +12·1° in H_2O) was dissolved in a warm solution of (±)-mandelic acid (2·040 g.; m. p. 118·5—119°) in alcohol (30 c.c.). After several days at the toom temperature the *salt* separated as needles; these were washed with ethyl alcohol [(yield, 1·065 g.; m. p. 156—157°) (Found: C, 54·8; H, 6·4. $C_6H_{14}O_2N_4,2C_8H_8O_3$ requires C, 55·2; H, 6·3%), and a portion (0·794 g.) was dissolved in N-hydrochloric acid. The solution, extracted with ether, gave 0·474 g. of mandelic acid [α_D + 0·04° for a solution (17·5 c.c.) in alcohol]. (Measurements of optical activity were made as described in Part I.) The alcoholic mother-liquor, when evaporated to dryness, afforded a residue, and this was digested with ether. The extract afforded 0·294 g. of mandelic acid with α_D −0·02°. The ether-insoluble residue (1·520 g.) dissolved in hydrochloric acid; the solution, extracted with ether, gave 0·910 g. of mandelic acid, α_D −0·03°. Both the needles and the ether-insoluble residue from the alcoholic mother-liquor contained 60·0% of mandelic acid as found by the ether-extraction procedure (Calc. for $C_6H_{14}O_2N_4,2C_8H_8O_3$: $C_8H_8O_3$, 63·6%). Similar results were obtained when 50% aqueous alcohol was substituted for alcohol.

(*b*) A solution (20·0 c.c.) of (±)-mandelic acid (1·500 g.) in alcohol was added to powdered anhydrous L-arginine (0·750 g.). At intervals 17·5 c.c. of the solution were removed, examined polarimetrically, and returned (see Table 1). The change in rotation at 64 hours coincided with

Table 1.

Time (hr.)	23	64	220	238
α	+0·18°	−1·01°	−2·51°	−2·53°

the separation of crystals on the surface of the L-arginine. After 238 hr. the suspension was filtered and the crystals (1·301 g.) were collected. They contained 0·635 g. of mandelic acid (Found: C, 62·7; H, 4·9; N, 0. Calc. for $C_8H_8O_3$: C, 63·2; H, 5·3%), α_D +2·47° as a solution (17·5 c.c.) in alcohol. The filtrate, evaporated to dryness, gave a residue and this was digested with ether. The soluble fraction was mandelic acid (0·794 g.), α_D −2·46° as a solution (17·5 c.c.) in alcohol. The insoluble portion (0·076 g.) was a salt containing 0·045 g. of mandelic acid (59%) and having α_D −0·15° as a solution (17·5 c.c.) in alcohol.

The (+)-form of mandelic acid combined preferentially in similar experiments with L-lysine instead of L-arginine, but no resolution was observed when the solid phase was L-glutamic acid, L-asparagine, L-tyrosine, L-alanine, or L-histidine.

L-Arginine Dimandelate and (±)-Mandelic Acid.—L-Arginine dimandelate was prepared from the components in alcohol (0·1954 g. of the salt gave 0·1140 g. mandelic acid having $[\alpha]_D$ +3·0°). The finely powdered salt (0·4054 g.) was kept in a stoppered flask for 8 days at 15° with a solution of (±)-mandelic acid in alcohol (20 c.c.). The undissolved crystals were collected and washed with ether (yield, 0·392 g.); they afforded 0·227 g. of mandelic acid, $[\alpha]_D$ +3·0°. The filtrate contained only inactive mandelic acid (0·4802 g.).

L-Arginine (−)-Mandelate and (±)-Mandelic Acid.—The powdered salt prepared from (−)-mandelic acid, $[\alpha]_D$ −160°, was repeatedly extracted with ether and then suspended for 24 hours in more dry ether. The medium remained optically inactive. The purified salt (0·3868 g.) was suspended at 15° in alcohol (20 c.c.) containing (±)-mandelic acid (0·4840 g.). At intervals 17·5 c.c. of the solution were removed, examined polarimetrically (see Table 2) and

Table 2.

Time (hr.)	45	24	73
α_D	−0·25°	−0·30°	−0·33°

then returned. After 8 days the salt was collected and washed with a small volume of ether. The filtrate and washings were concentrated, filtered, and finally evaporated to dryness. The residue was mandelic acid (0·421 g.; m. p. 118·5°; $[\alpha]_D$ −13°). The mandelic acid extracted from the salt had become correspondingly less lævorotatory ($[\alpha]_D$ −132°).

Replacement of (−)- by (+)-Mandelic Acid on Wool.—Wool fibres (50 g.; purified as described in Part I) were kept for 6 days at the room temperature in water (1250 c.c.) containing (−)-

mandelic acid (10 g.; $[\alpha]_D$ −160°). The wool was then removed, squeezed as free as possible from the medium, and immersed immediately in water (1250 c.c.) containing (±)-mandelic acid (10 g.). After 30 min. the wool was removed, squeezed, and finally kept for 4 days in a similar solution of (±)-mandelic acid. The solution then afforded 9·88 g. of mandelic acid, $[\alpha]_D$ −5·4°.

Relation between the Rates of Absorption and Resolution of Mandelic Acid on Wool.—Wool fibres were extracted (Soxhlet) with absolute alcohol for 24 hr., then with ether for the same period, and finally with alcohol for 8 hr. After being washed with water, they were dried in air and stored over anhydrous calcium chloride *in vacuo*.

Purified wool (30·0 g.) was immersed in distilled water at 31·3° and, when thoroughly wetted, was squeezed and then transferred to a solution of (±)-mandelic acid (5·000 g.) in water (500 c.c.) at 31·3°. At intervals the wool was collected, pressed in a coarse-mesh sintered-glass funnel, and thoroughly squeezed. The adsorbed mandelic acid was removed by treatment with dilute aqueous ammonia, and the weights and rotations of the absorbed and unabsorbed acid were determined. The time of immersion of the wetted wool in the aqueous acid was taken as zero, and the period of immersion as the time which elapsed until the wool was removed and one-half of the acid solution had been filtered. The filtration stage occupied 10—15 sec. in all. Each result recorded in Table 3 is the mean of several.

TABLE 3.

Time	Unabsorbed		Mandelic acid absorbed		Total accounted	Mandelic acid (%)	
(sec.)	(g.)	α_D	(g.)	α_D	for (g)	absorbed *	resolved *
30	3·667	−0·05°	1·017	+0·06°	4·684	49·9	25·0
75	3·447	−0·08	1·309	+0·09	4·756	64·3	37·5
105	3·321	−0·10	1·401	+0·10	4·722	68·8	41·7
225	3·206	−0·14	1·503	+0·14	4·709	73·8	58·3
420	3·172	−0·15	1·565	+0·15	4·717	76·8	62·5
660	3·012	−0·15	1·709	+0·16	4·721	83·9	66·7
950	2·962	−0·16	1·742	+0·17	4·704	85·5	70·8
1320	2·841	−0·17	1·871	+0·18	4·712	91·8	75·0
1800	2·802	−0·18	1·917	+0·19	4·712	94·8	79·5
5 days	2·679	−0·23	1·994	+0·24	4·673	100	100
6 days	2·651	−0·24	2·080	+0·24	4·731	100	100

* After 6 days.

TABLE 4.

Duration of contact (days)	5	5	10	10	15	15
Unabsorbed acid, g.	2·719	2·784	2·741	2·754	2·825	2·721
,, α_D	−0·22°	−0·23°	−0·24°	−0·23°	−0·23°	−0·24°

TABLE 5.

Contact	Acid desorbed		Acid combined with wool		Acid desorbed as % of acid desorbed after 15 days
(sec.)	(g.)	α_D	(g.)	α_D	
55	0·406	0·00°	1·627	+0·24°	55
90	0·485	−0·01	1·542	+0·24	66
180	0·551	−0·01	1·446	+0·25	75
420	0·676	−0·01	1·340	+0·24	92
670	0·718	−0·02	1·284	+0·26	98
5 days	0·761	−0·01	1·249	+0·24	
10 ,,	0·702	−0·01	1·273	+0·23	100
15 ,,	0·741	−0·01	1·260	+0·26	

Desorption of Mandelic Acid from Wool.—(*a*) Wool (30 g.) was brought into contact with a solution of mandelic acid (5 g.) in water (600 c.c.) at room temperature. The results in Table 4 were obtained. The wool containing the absorbed acid was immersed in water (600 c.c.) for a time. The solution was then filtered and the amount of acid remaining on the wool and the quantity desorbed were determined, together with the rotations of the two fractions (see Table 5).

(*b*) Wool (30 g., dry wt.) was brought into equilibrium with mandelic acid (5 g.) in water (600 c.c.) at room temperature; 2·525 g. of acid remained unabsorbed (α_D −0·26°). The wool was withdrawn, immersed in water (600 c.c.), and withdrawn after a time, and the immersion and removal repeated with fresh water (600 c.c.) at each stage. Results are in Table 6. 0·465 g. of acid remained absorbed (α_D +0·16°).

Effect of Previous Treatment with Mandelic Acid on the Selective Absorption of Mandelic Acid by Wool.—Wool (30 g., dry wt.) was immersed in a solution of mandelic acid (5 g.) in water (600 c.c.) for 5 days at the room temperature. The resulting solution was filtered and the absorbed acid removed from the wool by immersion in 1·5% aqueous ammonia (400 c.c.) for 10 min., filtration, reimmersion for the same time in 300 c.c. of a similar solution, and finally washing in

TABLE 6.

Desorption (hr.)	Acid desorbed (g.)	α_D	Total acid desorbed (% of total acid absorbed)
0·5	0·834	−0·02°	36
0·5	0·421	0·00	54
0·5	0·241	0·02	64
18·0	0·379	0·07	80

TABLE 7.

Cycle	Acid absorbed g.	α_D	Acid unabsorbed g.	α_D
First	2·394	0·25°	2·543	−0·25°
Second	2·382	0·24	2·522	−0·25
Third	2·351	0·25	2·574	−0·25

running water for 12 hours. The treated wool was brought into equilibrium with aqueous mandelic acid of the inital concentration, the system was analysed, and the sorption and desorption were repeated several times with the same sample of wool. The results shown in Table 7 were obtained. The rates of absorption and resolution of mandelic acid on the original sample of wool were compared with the corresponding rates on wool which had completed one cycle of sorption and desorption : for results see Table 8.

TABLE 8.

Contact (min.)	Acid absorbed (g.)	% of equilibrium amount	α_D	% of equilibrium value	Acid unabsorbed (g.)	α_D
Original wool.						
1	1·049	44	0·05°	19	3·851	−0·05°
3	1·644	68	0·10	38	3·270	−0·10
10	1·936	80	0·17	65	2·941	−0·18
5 days	2·418	100	0·26	100		
Mandelic acid-treated wool.						
1	1·025	43	0·05	20	3·805	−0·05
2	1·373	58	0·08	32	3·442	−0·08
5	1·730	73	0·12	48	3·191	−0·11
13	2·147	90	0·18	72	2·724	−0·18
5 days	2·366	100	0·25	100	2·532	−0·25

Selective Absorption of Mandelic Acid Derivatives.—The absorption of substituted mandelic acids, prepared by known methods, on wool was studied by the following method. 30 g. of dry wool were left in contact with an amount of acid approx. equivalent to 5 g. of mandelic acid, in a mixture of water (400 c.c.) and ethyl alcohol (300 c.c.), for 5 days at the room temperature. The solutions were evaporated at the room temperature under reduced pressure (but at 20—30° for the *o*-ethoxy-acid). Low-temperature evaporation was especially necessary with *o*-nitromandelic acid which developed a red colour when heated. Results are in Table 9.

TABLE 9

R in $C_6H_4R{\cdot}CH(OH){\cdot}CO_2H$	Wt. of acid (g.)	Acid absorbed g.	α_D	Acid unabsorbed g.	α_D
H	5·0	2·194	0·29°	2·707	−0·28°
o-NO_2	6·6	2·978	0·35	3·524	−0·32
m-NO_2	6·6	2·899	0·17	3·175	−0·18
p-NO_2	6·6	2·760	0·17	3·648	−0·17
p-OMe	6·0	2·471	0·24	3·310	−0·25
o-OEt	6·45	2·315	0·23	3·976	−0·22
p-OH (as monohydrate)	5·09	2·547	0·20	2·354	−0·22

m-(p-*Hydroxyphenylazo*)*mandelic Acid.*—Wool (50 g., dry wt.) was treated with *m*-(*p*-hydroxyphenylazo)mandelic acid (6·66 g.) (Brode and Adams, *J. Amer. Chem. Soc.*, 1926, **48**, 2202) in a mixture of water (500 c.c.) and ethyl alcohol (300 c.c.) for 5 days at room temperature.

The unabsorbed acid (1·277 g.) was recovered by evaporation at 30—40° under reduced pressure, and the absorbed acid (4·4 g.) by treatment with hot dilute aqueous ammonia. Solutions of both fractions were too deeply coloured for accurate measurement of optical rotation. Each was reduced, therefore, with sodium dithionite, but in neither case did the observed rotation of the resulting pale yellow solution exceed the limit of experimental error.

Absorption of Mandelic Acid by Silk Fibroin.—Degummed, unweighted, natural silk was extracted (Soxhlet) with alcohol and then ether, and washed with water. The silk (115 g., dry wt.) was then left in a solution of (±)-mandelic acid (10 g.) in water (800 c.c.) for 5 days at room temperature. The weight of acid absorbed was 4·885 g., and of acid unabsorbed 4·768 g. Both fractions were optically inactive.

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