SUMMARY

1. A method for the preparation of crystalline alcohol dehydrogenase from horse liver is described, which is simpler and milder than previous methods.

2. The product appears to be identical with that of the previous method, as judged by specific extinction at 280 m μ , specific activity and thiol group content, and is no more unstable than other preparations.

3. Maximum-rate data are reported and discussed. Discrepancies with the results of previous workers are attributed partly to the low specific activity of earlier preparations of the enzyme, and partly to the presence of an inhibitor in preparations of reduced diphosphopyridine nucleotide.

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Formation of Chromenols on Alumina from Quinones having an Isoprenoid Side Chain

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The extraction of ubichromenol from human-kidney unsaponifiable lipids and the elucidation of its structure have been described (Laidman, Morton, Paterson & Pennock, 1959, 1960). Ubichromenol from this tissue was shown to be a cyclic isomeride of ubiquinone (50). Links (1960) has suggested that ubichromenol is not a natural product but is an artifact of isolation formed by the action of alumina on ubiquinone. Links was able to make ubichromenol by adsorption of ubiquinone on alumina and elution with acetone-hydrochloric acid after 24 hr. The process has been repeated by Green, Edwin, Diplock & McHale (1960) and by Shunk *et al.* (1960). The last-named were able to show that the ubichromenol could be eluted with methanol-ether, that is, without using an acidified

eluent. We have also repeated Links's procedure on different ubiquinones and have also shown a conversion on acid-washed Brockmann Grade 2 alumina with the formation of a much cleaner product. Solanachromene (Rowland, 1958) has been produced by adsorption of koflerquinone (Kofler *et al.* 1959) on acid-washed alumina weakened with water. Vitamin K₁ has been subjected to a similar process.

EXPERIMENTAL

Materials. Ubiquinone samples and koflerquinone were kindly donated by Hoffmann-La Roche and Co. Ltd., Basle (through Drs O. Isler and U. Gloor). Vitamin K₁ was purchased from Roche Products Ltd., Welwyn Garden City, Herts. Alumina (Grade O) was purchased from P. Spence and Sons Ltd., Widnes, Lanes, magnesium oxide

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(for chromatographic adsorption analysis) from British Drug Houses Ltd. and silicic acid (100-mesh, analytical reagent) from Mallinckrodt Chemical Works (New York, U.S.A.).

Chromatography. Separation of ubiquinone and ubiquinone was carried out by chromatography on acid-washed Brockmann Grade 2 alumina. Grade O alumina was treated with N-HCl and washed with water to remove most of the chloride. The alumina was dried by heating to 115° overnight. The alumina was then partially deactivated with 2% of water to Brockmann Grade 2. The ubiquinone was eluted with 7% and 8% ether in light petroleum (b.p. $40-60^\circ$), and ubiquinone was eluted with 10-15% ether in light petroleum. Light petroleum (b.p. $40-60^\circ$) was dried over sodium and redistilled; diethyl ether was dried over sodium and redistilled over reduced iron before use.

Spectrophotometry. Ultraviolet-absorption spectra were measured with a Unicam SP. 500 photoelectric spectrophotometer. By ultraviolet absorption it was possible to estimate as little as $60\mu\text{g.}$ of ubiquinone and $110\mu\text{g.}$ of ubiquinone. Infrared-absorption spectra were measured with solvent-free films in a Perkin-Elmer Infracord spectrophotometer (model 137).

Conversion on Grade O alumina. The Links's procedure was repeated on ubiquinones (50), (45) and (30). The details for ubiquinone (30) are given below. 300 mg. of ubiquinone (30) was put on a column of 30 g. of Grade O alumina as purchased. As observed by Links, the colour of the adsorbed ubiquinone turned from yellow to brown. After 24 hr. in the dark, the column was washed with acetone-10% HCl (9:1, v/v). The solution was then diluted with water and extracted with ether. The red-orange extract was chromatographed on an acid-washed Brockmann Grade 2 alumina (100 g.) column. A small amount of ubiquinone was eluted with 7% ether in light petroleum, and 10% ether in light petroleum eluted a deep-red oil which showed an ultraviolet-absorption spectrum similar to that of ubiquinone. Further products were eluted with 20% ether in light petroleum, and with ether. Both fractions showed ultraviolet spectra which had resemblances to that of ubiquinone but the infrared-absorption spectra were clearly different.

Conversion on acid-washed alumina weakened with water. It was found that by leaving ubiquinone in contact with acid-washed Brockmann Grade 2 alumina, ubiquinone could be formed. The ubiquinone was adsorbed either on a column of alumina or on a similar weight of alumina in an Erlenmeyer flask. After a known time the products were then extracted from the alumina with ether and the extract was chromatographed on Brockmann Grade 2 alumina to separate the unchanged ubiquinone from the ubiquinone which had been produced.

Conversion on other adsorbents. The conversion of ubiquinone into ubiquinone was studied in a manner similar to that described above on Grade O alumina partially deactivated with water, and also on acid-washed Grade O alumina.

Action of alumina on vitamin K_1 and koflerquinone. The formation of chromenols from vitamin K_1 and koflerquinone was studied on acid-washed alumina deactivated with water. Vitamin K_1 was left on acid-washed Brockmann Grade 3 alumina overnight and koflerquinone was left on acid-washed alumina (partially deactivated with 10% of water) for 24 hr.

RESULTS

The results obtained when untreated Grade O alumina was used are given in Table 1.

Links (1960) found that if he used acid-washed alumina, or alumina with water added, the rate of conversion of ubiquinone into ubiquinone was decreased. Earlier work in this laboratory (Hemming, 1958) showed that 2 mg. of ubiquinone (estimated spectrophotometrically) could be chromatographed on 10 g. of acid-washed Brockmann Grade 3 alumina and recovered quantitatively. In view of Links's results it was decided to repeat this experiment on a larger scale so that if a very small conversion into ubiquinone occurred it could be detected.

Ubiquinone (50) (25 mg.) was chromatographed on 30 g. of acid-washed Brockmann Grade 2 alumina. Elution of the ubiquinone fraction was complete 1 hr. after the commencement of the chromatography. Spectrophotometric examination of the various fractions indicated that there was full recovery of ubiquinone, and no measurable amount of ubiquinone was produced. The experiment was repeated several times, the period of contact with the alumina being varied. The results are shown in Table 2.

The ubiquinone produced on acid-washed Brockmann Grade 2 alumina was pale yellow and appeared to be the only major selectively absorbing product eluted. Further work was carried out on

Table 1. *Production of ubiquinone on untreated Grade O alumina*

The ubiquinone was left on 30 g. of untreated Grade O alumina for 24 hr.

Material	Amount used (mg.)	Ubiquinone recovered* (%)	Ubiquinone recovered as ubiquinone* (%)
Ubiquinone (50)	500	10.5	21.3
Ubiquinone (45)	300	7.1	20.1
Ubiquinone (30)	300	3.2	24.1

* Spectrophotometric assay on chromatographic fraction.

Table 2. *Production of ubiquinone on acid-washed Brockmann Grade 2 alumina as influenced by time*

Alumina columns (30 g.) were used in each case.

Time on column (hr.)	Ubiquinone (50) used (mg.)	Ubiquinone recovered (%)	Ubiquinone recovered as ubiquinone (%)
$\frac{1}{2}$ -1	25.0	100	—
2-2 $\frac{1}{2}$	29.5	97.6	< 0.6
17	25.4	56.6	7.2
144	12.7	33.0	25.4

similarly standardized alumina but the adsorption was effected in conical flasks rather than in chromatography columns. This allowed all the alumina to be used in the initial adsorption of the ubiquinone (Table 3).

When ubiquinone was left on acid-washed Grade O alumina little ubiquinone was formed and no ubiquinone was recovered. Non-acid-washed alumina, partially deactivated with water, gave lower yields of ubiquinone than did acid-washed Brockmann Grade 2 alumina. The recoveries of ubiquinone were also lower. Ubiquinone left in contact with MgO was completely destroyed after 24 hr. and no ubiquinone was produced. However, there was no conversion or destruction of ubiquinone even after 4 days' contact with silicic acid.

Samples of ubiquinone (50), (45) and (30) were made by adsorbing the corresponding ubiquinone on acid-washed Brockmann Grade 2 alumina in an Erlenmeyer flask. After 2½ days the alumina was extracted with ether and ubiquinone was separated from residual ubiquinone by chromatography on alumina. The ubiquinone was purified by further chromatography and a sample of the acetate was prepared (Laidman *et al.* 1960) in each case. The ultraviolet-absorption spectra of the three ubiquinone and their acetates were qualitatively identical with those of 'natural' ubiquinone and its acetate respectively. The molecular extinction coefficients (ϵ) of ubiquinone were within 5% of each other (see Table 4). The infrared-absorption spectra of the

three synthetic ubiquinone were qualitatively identical with that of ubiquinone from human kidney (Laidman *et al.* 1960).

Formation of solanachromene. Koflerquinone (21.7 mg.) was put on a column of 20 g. of acid-washed Brockmann Grade 2 alumina and left for 24 hr. After this time the contents of the column were washed with 2, 4, 6, 8, 10 and 20% ether in light petroleum and finally ether. The 2% ether in light petroleum fraction, which would normally contain the koflerquinone, showed little of spectrophotometric interest. The 10% ether in light petroleum fraction contained a substance which exhibited peaks in the ultraviolet region at 233, 267 and 332 m μ , with a shoulder at 275 m μ . This compound proved to be solanachromene. From spectrophotometric data it was estimated that 5.2% of the koflerquinone had been recovered in the form of solanachromene.

As the koflerquinone had been destroyed to such a large extent, a similar experiment was carried out with alumina deactivated still further. Koflerquinone (27.5 mg.) was adsorbed on 20 g. of acid-washed alumina partially deactivated with 10% of water (2% of water added to the same alumina made Brockmann Grade 2). The koflerquinone was left in contact with the alumina for 6 hr., whereupon the alumina was extracted with ether. The extract was then chromatographed on a column of 20 g. of Brockmann Grade 2 alumina; 2% ether in light petroleum eluted 21.1 mg. of koflerquinone, i.e. 80.2% of the original material was recovered; 8 and 10% ether in light petroleum eluted 2.25 mg. of solanachromene, i.e. equivalent to 8.2% of the original koflerquinone. In another experiment, koflerquinone was left on acid-washed alumina, partially deactivated with 8% of water, for 17½ hr. The yield of solanachromene was 9.6% but the recovery of unchanged koflerquinone was only 30.3%.

The solanachromene from these experiments was bulked and acetylated as for ubiquinone (Laidman *et al.* 1960). The solanachromene acetate was chromatographed on acid-washed Brockmann Grade 2 alumina and eluted with 2% ether in light petroleum. The ultraviolet-absorption spectrum of this material was identical with that described by Rowland (1958), showing $\lambda_{\max}$ 229, 236, 267 and 317 m μ , with shoulders at 276 and 330 m μ . Minima were present at 225, 234, 252 and 293 m μ . Mild saponification of this material followed by chromatography on alumina gave a sample of solanachromene which showed $\lambda_{\max}$ 233, 267 and 332 m μ , with a shoulder at 276 m μ and $\lambda_{\min}$ at 227, 248 and 295 m μ . This absorption spectrum appears identical with that described by Rowland (1958) and with the actual absorption curves kindly sent to us by Dr R. L. Rowland. By comparison with

Table 3. *Formation of ubiquinone on acid-washed Brockmann Grade 2 alumina*

Ubiquinone (50) adsorbed from light petroleum on 20 g. of alumina in an Erlenmeyer flask.

Time on alumina (days)	Ubiquinone used (mg.)	Ubiquinone recovered (%)	Ubiquinone recovered as ubiquinone (%)
1	17.9	88.7	9.2
2	17.9	78.5	14.2
3	17.4	52.1	21.1
5	18.2	31.0	25.5

Table 4. *Ultraviolet absorption of synthetic ubiquinone and its acetate*

Values given are mean values for chromatographically pure samples of ubiquinone (50), (45) and (30).

Ubiquinone		Ubiquinone acetate	
$\lambda_{\max}$ (m μ)	ϵ	$\lambda_{\max}$ (m μ)	ϵ
233	17 450	231	20 830
275	7 720	276	8 340
283	7 310	285	7 680
332	3 230	316	2 630

the values given by Rowland, our sample of solanachromene appears to be 80 % pure.

Action of alumina on vitamin K₁. Vitamin K₁ (20.7 mg.) was adsorbed on 20 g. of acid-washed Brockmann Grade 2 alumina and left for 17½ hr. After extraction with ether, the products were chromatographed on acid-washed Brockmann Grade 2 alumina. The following fractions were collected: light petroleum, 1, 2, 10, 20 % ether in light petroleum and pure ether eluates. Of vitamin K₁, 93.9 % (19.4 mg.) was recovered in the 1 % ether in light petroleum fraction and 20 % ether-light petroleum eluted a small amount of material which showed in the ultraviolet region $\lambda_{\max}$. 250 and 327 m μ , $\lambda_{\min}$. 230 and 314 m μ and an inflexion at 285–290 m μ . A similar product was obtained with acid-washed alumina partially deactivated with 10 % of water. Tishler, Fieser & Wendler (1940) synthesized naphthathocopherol by cyclization of vitamin K₁ quinol. This compound, a chromenol, showed absorption in the ultraviolet region with $\lambda_{\max}$. 247 and 320 m μ . It seems possible that the material we have obtained is the related chromenol.

DISCUSSION

We have been able to reproduce the results obtained by Links with untreated Grade O alumina. As a method of obtaining pure ubichromenol this method has disadvantages. At least two other ubichromenol-like substances are produced; the ubichromenol fraction is highly coloured and difficult to purify, and there is low recovery of unchanged ubiquinone.

By the use of acid-washed Brockmann Grade 2 alumina with more prolonged times of contact it was possible to obtain yields of ubichromenol as high as 25 %. Ubichromenol is the only major product and the fraction is pale yellow like the natural compound. Moreover, there is a high recovery of unchanged ubiquinone. For example, after 2 days it is possible to recover 78 % of the ubiquinone unchanged and 14 % as ubichromenol. It is apparent from these results that ubichromenol can best be synthesized from ubiquinone by leaving the latter in contact with acid-washed Brockmann Grade 2 alumina for 2–3 days. The unchanged ubiquinone can be used again for further conversion. The ubichromenol produced in this manner appears to be identical with that isolated from human kidney, but by this chemical conversion a racemic mixture is formed, whereas the ubichromenol from human kidney has been shown to be optically active (– 50° at 430 m μ) (Morton, 1960).

The chromenols of koflerquinone and vitamin K₁ can be made from the quinones on acid-washed Grade O alumina partially deactivated with water. The conversion of these quinones into chromenols seems to go less readily than with ubiquinone. Rowland (1958) suggested that solanachromene had the formula C₅₈H₈₈O₂. Rowland & Giles (1960) have now shown that the correct formula for solanachromene is C₅₃H₈₀O₂, as would be expected by cyclization of koflerquinone.

SUMMARY

1. The work of Links (1960) has been repeated. Ubiquinone left in contact with Grade O alumina overnight is partially converted into ubichromenol and at least two other products. The ubichromenol fraction thus made is highly coloured and difficult to purify.

2. By adsorbing ubiquinone on acid-washed Brockmann Grade 2 alumina for 2–3 days, ubichromenol can be formed with no other major products, and the recovery of unchanged quinone is high. The ubichromenol is similar in colour to the natural material and is relatively easy to purify.

3. Solanachromene has been made by adsorbing koflerquinone on acid-washed Grade O alumina partially deactivated with 10 % of water.

4. Adsorption of vitamin K₁ on acid-washed Brockmann Grade 2 alumina gave a product which may be a chromenol related to naphthathocopherol.

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