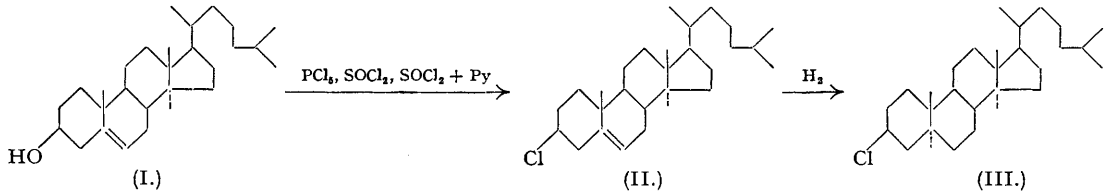
254. *Steroids and the Walden Inversion. Part II. Derivatives of Δ^5-Cholestene and Δ^5-Androstene.*

By C. W. Shoppee.

On the basis of the constitutions assigned in Part I, cholesteryl chloride is shown to be 3(β)-chloro-Δ^5-cholestene. The steric orientation of substitution reactions at C_3 in Δ^5-steroids is discussed; in striking contrast to the general occurrence of inversion in the replacement reaction ·Cl ⟶ ·OR and the occurrence of inversion or retention according to the orientation rules of Cowdrey *et al.* (*J.*, 1937, 1252) for the substitution ·OR ⟶ ·Cl in the saturated series, both these types of replacement take place in the Δ^5-series with preservation of configuration and irrespective of the substituting agent within the range of reagents and conditions examined. It is suggested that the retention of configuration characteristic of the substitution reactions at C_3 of Δ^5-steroids is due to polarisation of the C_5:C_6-double bond, the electrons of which interact with the cationic charge formed at C_3 by ionisation of the group substituted to produce a C_3–C_5 bond of mixed covalent–electrovalent type; this binding leads to a pyramidal configuration for the transition state and so to preservation of configuration.

On this basis, a consistent picture is obtained for substitution reactions at C_3 in the Δ^5-steroid series, which harmonises with the configurations assigned (Part I) in the saturated series.

In Part I (preceding paper), arguments were adduced to show that the so-called " α "-cholestanyl chloride, m. p. 115°, has the constitution 3(β)-chlorocholestane (III). When cholesterol (I) is treated with phosphorus pentachloride (Planer, *Annalen*, 1861, **118**, 25; Loebisch, *Ber.*, 1872, **3**, 510; Mauthner, *Monatsh.*, 1894, **15**, 87), or with thionyl chloride alone (Diels, Abderhalden, and Blumberg, *Ber.*, 1904, **37**, 3092; 1911, **44**, 287) or in presence of pyridine (Daughensbaugh and Allison, *J. Amer. Chem. Soc.*, 1929, **51**, 3665), high yields of one and the same product, cholesteryl chloride, m. p. 96°, are obtained. Since catalytic hydrogenation of cholesteryl chloride affords 3(β)-chlorocholestane (III) (Mauthner, *Monatsh.*, 1909, **30**, 635; Marker, Whitmore, and Kamm, *J. Amer. Chem. Soc.*, 1935, **57**, 2358) in quantitative yield (*vide infra*), cholesteryl chloride is to be regarded as 3(β)-chloro-Δ^5-cholestene (II). Crystallographic evidence also supports this view: " from

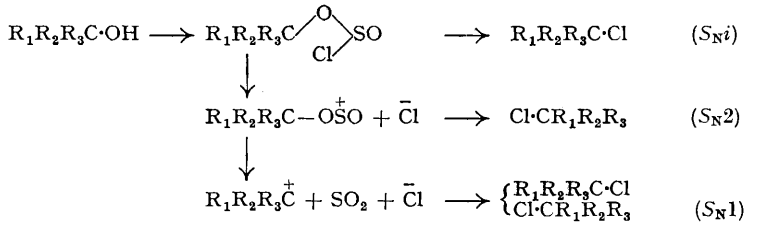


(I.) (II.) (III.)

the crystal unit cell dimensions and even more from the intensities of the *c* plane reflexions, it is clear that " α "-cholestanyl chloride has the same configuration (at C_3) as cholesteryl chloride " (Crowfoot, " Vitamins

and Hormones," New York, 1944, Vol. 2, p. 450). The hydroxyl group of cholesterol thus consistently undergoes replacement by chlorine with retention of configuration.

This relationship is unusual, inasmuch as although the three reagents specified can yield the same stereochemical product (*e.g.*, with β-*n*-octyl alcohol, ethyl lactate), they then cause inversion (mechanisms S_N1, S_N2). Retention of configuration according to the concepts laid down by Ingold *et al.* (*J.*, 1937, 1252) and recently summarised by Dostrovsky, Hughes, and Ingold (this vol., p. 186 *et seq.*) requires an intramolecular mechanism (S_Ni), leading to a transition state of pyramidal type, which is promoted by electron releasing groups and under conditions inhibitory to ionisation; retention of configuration with employment of all the three reagents specified is rational only if the structural influences favour mechanism S_Ni sufficiently, and would be the logical outcome of very powerful electron release—a circumstance which from inspection of the formula of cholesterol (I) would not be expected to arise.

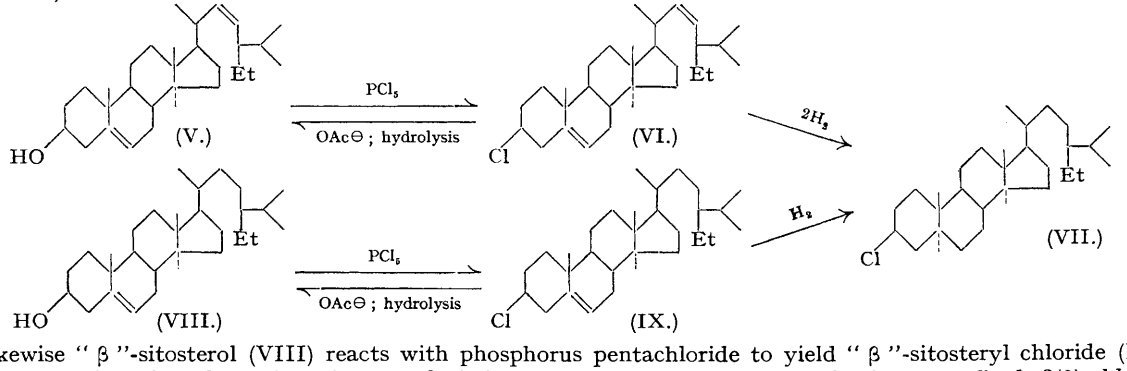


This singular behaviour of the Δ^5-cholestene system is not confined to the substitution of OR by Cl, but also appears in the replacement of Cl by OR. When cholesteryl chloride (II) is treated with acetate ions in acetic acid at 100°, the product is almost pure cholesteryl acetate (IV) (cf. Bergmann, *J. Amer. Chem. Soc.*, 1938, **60**, 1996), and hydrolysis furnishes a 91% overall yield of cholesterol * (I), unaccompanied by *epi*-cholesterol:

$$\text{(II)} \xrightarrow{\text{OAc}\ominus} \text{(IV; = II with OAc for Cl)} \xrightarrow{\text{OH}\ominus} \text{(I)}$$

Thus here substitution occurs with apparently complete retention of configuration, and in sharp contrast with the corresponding substitution reactions of the cholestanyl chlorides, and secondary alkyl halides generally, which proceed (mechanisms S_N1, S_N2) with substantially complete inversion of configuration; and it is difficult to envisage the operation of some internal rearrangement mechanism analogous to S_Ni in an essentially ionic reaction such as the conversion of (II) to (IV) by acetate ions in an ionising medium.

A similar situation exists in the relations subsisting between other Δ^5-sterols and their 3-chloro-derivatives. Thus stigmasterol (V) by treatment with phosphorus pentachloride undergoes substitution with retention of configuration to furnish stigmasteryl chloride, which must possess the 3(β)-chloro-constitution (VI) because catalytic hydrogenation affords 3(β)-chlorostigmastane [3(β)-chlorositostane] (VII) (cf. Part I, *loc. cit.*). Treatment of stigmasteryl chloride (V) with acetate ions in acetic acid at 100°, with subsequent alkaline hydrolysis, regenerates stigmasterol (IV) with retention of configuration (Marker and Lawson, *J. Amer. Chem. Soc.*, 1937, **59**, 2711):



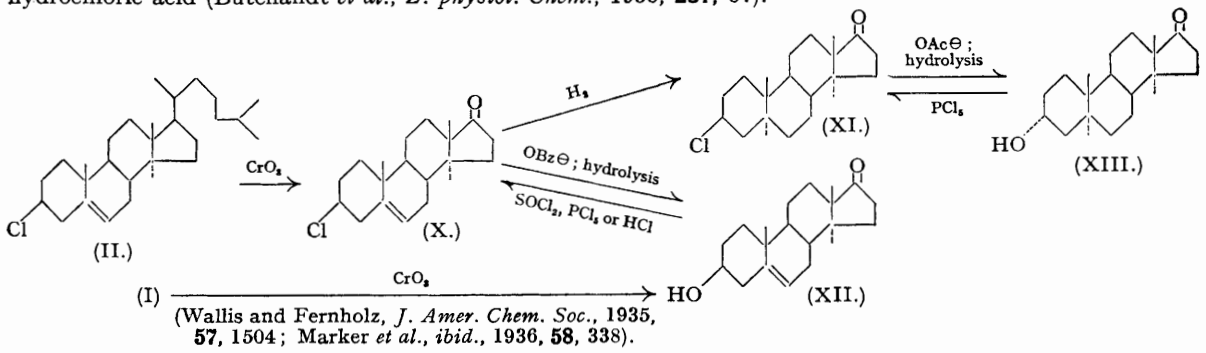
Likewise " β "-sitosterol (VIII) reacts with phosphorus pentachloride to yield " β "-sitosteryl chloride (IX) with retention of configuration, since catalytic hydrogenation of the last-named substance affords 3(β)-chlorostigmastane (VII); whilst treatment of (IX) with acetate ions in acetic acid at 100° and subsequent alkaline hydrolysis regenerates " β "-sitosterol with retention of configuration (Marker and Lawson, *loc. cit.*).

Once again, a similar position is disclosed amongst derivatives of Δ^5-androstene. When cholesteryl chloride (II) is oxidised (as the dibromide) with chromium trioxide (Marker *et al.*, *J. Amer. Chem. Soc.*, 1936, **58**, 338) " α "-chloroandrostenone, m. p. 157°, is obtained; this must be regarded as 3(β)-chloro-Δ^5-androsten-17-one (X),† since (i) catalytic hydrogenation converts it into 3(β)-chloroandrostan-17-one (XI) (cf. Part I,

* The statement of Marker, Kamm, Fleming, Popkin, and Wittle (*J. Amer. Chem. Soc.*, 1937, **59**, 619) which is not supported by experimental evidence is thus confirmed.

† This compound has no androgenic activity in the comb test (Ruzicka, Goldberg, and Wirz, *Helv. Chim. Acta*, 1935, **18**, 998).

loc. cit.) and (ii) it is obtained from 3(β)-hydroxy-Δ⁵-androsten-17-one (dehydro*iso*androsterone) (XII) not only by treatment with thionyl chloride—a reagent which tends to substitute with retention of configuration—but also by use of phosphorus pentachloride (Wallis and Fernholz, *J. Amer. Chem. Soc.*, 1937, **59**, 764) and of hydrochloric acid (Butenandt *et al.*, *Z. physiol. Chem.*, 1935, **237**, 57).*



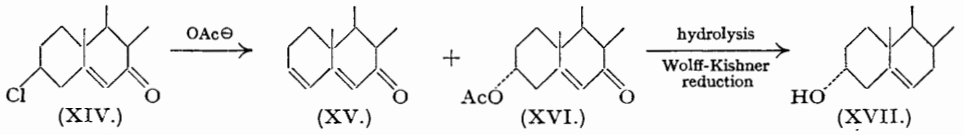
(Wallis and Fernholz, *J. Amer. Chem. Soc.*, 1935, **57**, 1504; Marker *et al.*, *ibid.*, 1936, **58**, 338).

In striking contrast to the reaction of (XI) with acetate ions in acetic acid, which proceeds with inversion to yield androsterone acetate and, after hydrolysis, androsterone (XIII)—a reaction which can be reversed by use of phosphorus pentachloride, a reagent which tends to substitute with inversion of configuration—3(β)-chloro-Δ⁵-androsten-17-one (X) reacts with benzoate ions in molten benzoic acid to give, after hydrolysis, 3(β)-hydroxy-Δ⁵-androsten-17-one (dehydro*iso*androsterone) (XII).

The situation outlined above may be summarised as follows. Of the replacement reactions at C_3: (*a*) $C{\cdot}OH \xrightarrow{SOCl_2} C{\cdot}Cl$, (*b*) $C{\cdot}OH \xrightarrow{PCl_5} C{\cdot}Cl$, (*c*) $C{\cdot}Cl \xrightarrow{OAc\Theta} C{\cdot}OAc$, in the saturated series (*a*) takes place with retention and (*b*) and (*c*) with inversion of configuration, but in the Δ⁵-series all three reactions (*a*), (*b*), and (*c*) take place with retention of configuration. Whilst a reaction mechanism (S_Ni) is available to account for retention in (*a*), and a similar mechanism would be possible in the presence of groups able strongly to influence the geometry of the transition state (cf. the case of α-phenyl-*n*-amyl alcohol which reacts with phosphorus pentachloride with retention of configuration) but is therefore improbable here in respect of retention in (*b*), it appears impossible to invoke some analogous internal rearrangement mechanism in regard to retention in (*c*).

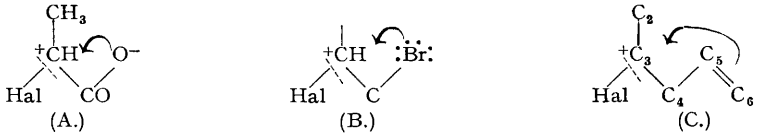
The sole constitutional feature distinguishing cholesterol and 3(β)-hydroxycholestanol, and the similar pairs of compounds referred to above, is the $C_5{:}C_6$-double bond. The incorporation of a double bond in a *cyclo*paraffin ring necessitates that the doubly-bound carbon atoms and those adjacent to them (C_4, C_7, C_{10}) lie in a plane, and that the angle $C_4{-}C_5{-}C_{10}$ approximates to 120°. The influence of such a structural modification is to compel rings A and B to conform more nearly to flat hexagons in cholesterol than in 3(β)-hydroxycholestanol; the general effect will be to flatten further the already nearly flat steroid nucleus, and this has been confirmed crystallographically (Crowfoot, *op. cit.*, p. 422). But since it has been shown (Part I) that the approximately planar character of the steroid nucleus facilitates the formation of a transition state of linear, as opposed to pyramidal, type and hence tends to lead to replacement with inversion of configuration, the geometrical modification arising from the introduction of a $C_5{:}C_6$-double bond can have no part in the consistent retention of configuration exhibited in the substitution reactions of compounds of the Δ⁵-series.

The conclusion is inescapable that the apparently abnormal behaviour of cholesterol and its Δ⁵-analogues must be ascribed to the polarisability of the $C_5{:}C_6$-double bond. In support of this is the interaction known to occur between C_3 and C_5 in the production of derivatives of *i*-cholestane, *i*-stigmastane, and *i*-androstane. Further, the qualitatively increased reactivity displayed by the 3-chlorine atom in cholesteryl, stigmasteryl, and sitosteryl chlorides as compared with the cholestanyl and stigmastanyl chlorides must be attributed to electron-release by the $C_5{:}C_6$-double bond, and an indication that this is so is provided by 7-ketocholesteryl chloride (XIV); Marker *et al.* (*J. Amer. Chem. Soc.*, 1937, **59**, 619) note with some surprise that the chlorine atom in this compound exhibits the qualitatively lesser reactivity characteristic of the cholestanyl and stigmastanyl chlorides, a circumstance which clearly arises from the enforced polarisation of the double bond by the 7-carbonyl group, $C_5{=}C_6{-}C_7{=}O$, in the direction opposite to that required to facilitate separation of the 3-chlorine atom. If this is true, then substitution of the 3-chlorine atom in (XIV) must proceed with inversion of configuration; and it is found (Marker *et al.*, *loc. cit.*) that the reaction of (XIV) with acetate ions in *n*-valeric acid furnishes 7-ketocholesterylene (XV) together with an acetate (XVI) which, after hydrolysis and reduction of the 7-keto-group, affords *epi*cholesterol (XVII); thus here inversion of configuration occurs in the manner characteristic of the saturated series.



* The polar influence of the 17-carbonyl group at C_3 is assumed to be negligible.

The suggestion that the retention of configuration uniformly observed in replacements at C_3 in the Δ^5-series is connected with the availability of electrons at C_3 arising from the polarisability of the C_5:C_6-double bond leads naturally to comparison with the first-order substitution reactions of the α-bromopropionate ion (Cowdrey, Hughes, and Ingold, *J.*, 1937, 1208). At this stage during private discussion with Professor Ingold the writer's attention was directed to a series of papers by Winstein *et al.* (*J. Amer. Chem. Soc.*, 1939, **61**, 1576, 1581, 1635, 2845; 1942, **64**, 2780, 2787, 2791, 2792, 2796; 1943, **65**, 613, 2196; 1946, **68**, 119) which show that the unshared electrons of an α-bromine atom are implicated in substitution reactions occurring at C_β in the system C_β–C_α·Br. It appeared therefore that the three examples (A), (B), and (C) illustrate essentially analogous processes.



In regard to case (A), Cowdrey, Hughes, and Ingold (*loc. cit.*) showed by a kinetically controlled stereochemical examination that the first-order hydroxylation and methoxylation reactions of the α-bromopropionate ion are unimolecular and take place with 90—100% retention of configuration by mechanism S_N1 without detectable racemisation. In discussing this result, Cowdrey, Ingold, Hughes, Masterman, and Scott (*J.*, 1937, 1252, especially pp. 1256 and 1261) point out that it is in conformity with theory since, owing to the anionic charge, the most stable configuration of the intermediate carbon cation is now of the pyramidal type, which corresponds to retention of stereochemical form.

Winstein, Lucas, and their collaborators (*locc. cit.*) have adduced evidence to show that OH, OAc, and OMe groups, and bromine atoms, participate in replacement reactions at an adjacent saturated carbon atom. They have discovered a case of substitution involving an intermediate cation preserved as to configuration by an α-bromine atom; they formulate an intermediate bromonium ion, using the covalent extreme for a link which Cowdrey *et al.* regard as largely electrovalent; but this makes no essential difference to the stereochemical interpretation (cf. Bateman, Church, Hughes, Ingold, and Taher, *J.*, 1940, 1010).

In regard to case (C), examination of the product of the substitution of the chlorine atom in cholesteryl chloride by acetate ions in acetic acid at 100° discloses 91% retention and no detectable amount (after hydrolysis) of *epi*cholesterol, and hence no detectable racemisation. The mechanism of substitution therefore appears to be exclusively S_N1, for although mechanism S_N2 was shown to operate in the case of the α-bromopropionate anion for both hydroxylation and methoxylation, this mechanism furnished 80—100% inversion of configuration, and so appears qualitatively to be excluded here. If the suggested operation of mechanism S_N1 may be generalised for other replacement reactions in the Δ^5-series we have the following picture: as the ionisation of the group to be replaced passes over its energy barrier and before the reagent intervenes, the polarisable electrons of the C_5:C_6-double bond interact with the carbon cation sufficiently powerfully to overcome both the energetic and the geometrical factors, which normally operate to favour production of a transition state of linear type, and to lead to the formation of a transition state of pyramidal type, the consequence of which is retention of configuration following attack of the reagent and completion of the reaction.

The foregoing analysis of the facts provides a consistent picture of replacement reactions at C_3 in the Δ^5-steroid series (case C) resting on circumstantial evidence, but no proof that the interpretation suggested is correct. An examination of the kinetics and steric orientation of nucleophilic substitutions in the simpler aliphatic analogues of case (C)—the methylallylcarbinyl and benzylmethylcarbinyl series—is being undertaken by Hughes and Ingold; and the writer hopes to make a kinetic study of some suitable replacement reaction (hydrolysis, alcoholysis, or acetolysis) of cholesteryl chloride. It is also intended to examine the chemistry of *epi*cholesterol and *cis*-dehydroandrosterone, and of *i*-cholestane and *i*-androstane derivatives, from the point of view now developed.

Experimental.

(All m. ps. were determined thermoelectrically on a Kofler block and are therefore corrected; limit of error ±2°.)

Acetolysis of Cholesteryl Chloride.—Cholesteryl chloride (250 mg., chromatographically purified and recrystallised from acetone, m. p. 96°), 2 c.c. of a 20% solution of freshly fused potassium acetate in pure acetic acid, and sufficient pure acetic acid (redistilled over chromium trioxide) to give a homogeneous mixture, were heated with exclusion of moisture for 4 hours at 100°. After removal of most of the acetic acid under reduced pressure, water was added, and the product extracted with ether. The ethereal extract was washed with water, sodium bicarbonate solution, and again with water, dried (Na_2SO_4), and evaporated. The residue (263·5 mg.) was completely crystalline, had m. p. 112—114°, and appeared to be pure cholesteryl acetate. To confirm this, the product was heated under reflux for 0·5 hour with 4% methyl alcoholic potassium hydroxide (2·5 c.c. ≡ approx. 3 mols.); working up furnished apparently pure cholesterol, m. p. 147—148° (235 mg.), which was dissolved in methanol (7 c.c.) and treated with a solution of digitonin (700 mg.) in warm methanol (7 c.c.). Addition of water (1·4 c.c.) gave a large precipitate; the mixture was kept for 1 hour at 0°, and then separated by centrifuging. The precipitate was twice mixed with methanol and re-separated by centrifuging.

The digitonide was dried in a desiccator, dissolved in dry pyridine (4 c.c.), and decomposed by addition of dry ether (40 c.c.); the precipitated digitonin was filtered off and washed with ether, and the filtrate evaporated to yield cholesterol (217 mg.; yield, 91%), m. p. 148° after crystallisation from methanol.

The methyl alcoholic filtrates were combined and evaporated completely under reduced pressure, and the residue was extracted with boiling dry ether (5 portions of 20 c.c.); these extracts by evaporation gave a little oil (9 mg.), which was distilled at 140°/0·001 mm., and an attempt was made to crystallise the distillate from methanol. A trace of

crystalline material, m. p. 80—90° (indefinite), was obtained; it gave a positive Beilstein test for halogen and possibly consisted essentially of unchanged cholesteryl chloride.

The yield of cholesterol was 91%, that of the digitonin non-precipitatable material 3·8%; losses, which appear to be unavoidable in digitonin separations, thus amounted to about 5%.

3(β)-*Chlorocholestane from Cholesteryl Chloride.*—Cholesteryl chloride, m. p. 96° (1·00 g.), was reduced by shaking with Adams's catalyst (100 mg.) in hydrogen at atmospheric pressure for 48 hours; the solvent used was pure ethyl acetate (10 c.c.) containing pure acetic acid (0·10 c.c.) (cf. Marker, Whitmore, and Kamm, *J. Amer. Chem. Soc.*, 1935, **57**, 2358). The product obtained by filtration from the catalyst and complete evaporation had m. p. 111—112° (1·00 g.); recrystallisation from acetone afforded prisms, m. p. 112°, $[\alpha]_D^{21°}$ +18°±2° (*c*, 1·809 in chloroform), which gave a pale but distinct yellow colour with tetranitromethane. Three recrystallisations from acetone gave a product, m. p. 112—113°, $[\alpha]_D^{21°}$ +21°±2° (on material dried at 100°/10 mm.; *c*, 1·635 in chloroform), which still gave a pale yellow colour with tetranitromethane and so still presumably contained traces of unreduced cholesteryl chloride, $[\alpha]_D^{20°}$ −27° (Beynon, Heilbron, and Spring, *J.*, 1936, 907). Part of the product (330 mg.) was therefore dissolved in pure acetic acid (4 c.c.) and treated with a 2% solution of chromium trioxide in acetic acid (4 c.c. ≡ 1·5 atoms of oxygen) at 60° for 0·5 hour with efficient stirring (cf. Ruzicka, Goldberg, and Brungger, *Helv. Chim. Acta*, 1934, **17**, 1389). After working up, the neutral material was dissolved in pentane and purified from neutral oxidation products by filtration through a column of aluminium oxide (Merck–Brockmann, activity III—IV: 10 g.) prepared in pentane. After recrystallisation from acetone the pure chloro-compound had m. p. 114—115°, $[\alpha]_D^{20°}$ +27°±1° (*c*, 1·911 in chloroform).

The author gratefully acknowledges the support of the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund, and the Anna Fuller Fund.

THE CHESTER BEATTY RESEARCH INSTITUTE,
THE ROYAL CANCER HOSPITAL (FREE), LONDON, S.W.3.

[*Received, August 8th*, 1946.]