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THE SYNTHESIS OF DIASTEREOMERIC EPOXY-(β-D-GLUCOPYRANOSYL)ETHANES AND 1,2-EPOXY-3-(β-D-GLUCOPYRANOSYL)PROPANES, AS IRREVERSIBLE INHIBITORS OF β-D-GLUCOSIDASE

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ABSTRACT

Diastereomeric epoxy-(β-D-glucopyranosyl)ethanes and 1,2-epoxy-3-(β-D-glucopyranosyl)propanes were synthesized to study the active site of β-D-glucosidases The mixtures of the diastereomeric epoxides of β-D-glucopyranosylethene and of 2-(β-D-glucopyranosyl)-1-propene tetraacetates were prepared by reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with the appropriate Grignard reagents, followed by oxidation with peroxyphthalic acid in dichloromethane Chromatographic separation of these mixtures, followed by deacetylation of each epoxide resulted in two pairs of diastereomers that inactivated irreversibly sweet-almond β-D-glucosidase

INTRODUCTION

Irreversible inhibitors have been extensively used in the study of the active site of enzymes[1] However, only a few investigations of irreversible inhibition of carbohydrases (hydrolases of *O*-glycosyl compounds, EC 3 2 1) have been reported, for example, the study by Legler *et al* [2] of the irreversible inhibition of β-D-glucosidases of different origin by conduritol B derivatives, the alkylation of the Asp-52 residue of lysozyme with 2,3-epoxypropyl 2-acetamido-2-deoxy-β-D-glucopyranosides[3] by Sharon *et al* [4], and the irreversible inhibition of *E coli* β-D-galactosidase with *N*-bromoacetyl-β-D-galactopyranosylamine by Yariv *et al* [5]

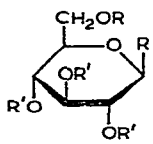
The difficulties in developing methods of study of the active site of definite carbohydrases by use of irreversible inhibitors are due mainly to the complexity of the syntheses Nevertheless, the chemical identification of the catalytic groups of enzymes requires a widening range of such inhibitors

The present paper reports the synthesis of irreversible inhibitors of β-D-glucosidase, namely *C*-glucopyranosyl derivatives having an alkylating group either in the immediate proximity of [diastereomeric epoxy(β-D-glucopyranosyl)ethanes, **5** and **6**] or distant from the sugar residue [diastereomeric 1,2-epoxy-3-(β-D-gluco-

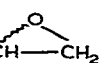
pyranosyl)propanes, **13** and **14**] The epoxide group was selected because it is capable of reacting with the carboxylate anion and the nondissociated carboxyl group[6], which are the catalytic groups in the active site of β-D-glucosidases of different origin, as shown by indirect evidence[2 7].

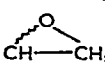
RESULTS AND DISCUSSION

The starting material for the epoxides **5** and **6**, (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)ethene (**1**), was initially synthesized by Zhdanov *et al*[8] by treatment of vinyl magnesium bromide with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide However, the compound obtained was not isolated in a pure state and the β-D-configuration of the *C*-glucosyl bond was not proved We have modified the synthesis[8] of **1** and isolated by chromatography in a yield of 30–35%, pure **1**, which was subsequently deacetylated into β-D-glucopyranosylethene (**2**)



1 R = CH=CH$_2$ R′= Ac
2 R = CH=CH$_2$, R′= H
3 R = CHO, R′= Ac
4 R = CH$_2$OAc, R′= Ac

5,6 R =  R′= H

7,8 R =  R′= Ac

9 R = CH$_2$CH=CH$_2$, R′= Ac
10 R = CH$_2$CH=CH$_2$, R′= H

11,12 R =  R′= Ac

13,14 R =  R′= H

15 R = CH$_2$CH$_3$, R′= Ac
16 R = CH$_2$CH$_2$CH$_3$, R′= Ac

Since neither optical rotation (because of the proximity of the double bond to the anomeric centre) nor the i r and p m r data give an unambiguous proof of the β-D configuration of a *C*-glucosyl bond, the structure of **1** was proved by chemical degradation Compound **1** was oxidized with osmic anhydride and sodium periodate into aldehyde **3**, which was reduced by sodium borohydride and acetylated to give the optically inactive acetate **4**, identical with 1,3,4,5,7-penta-*O*-acetyl-2,6-anhydro-D-*glycero*-D-*gulo*-heptitol[9]

Oxidation of **2** with peroxyphthalic acid was reported[10] to give a mixture of **5** and **6** We have found now that the mixture of the diastereomeric epoxide acetates **7** and **8**, formed by oxidation of **1**, can be separated by chromatography, and thus the individual diasteromers **5** and **6** obtained

The diastereomeric epoxides **13** and **14**, in which the side-chain oxide ring is separated from the sugar ring by one methylene group, were synthesized in a way similar to that just described The starting allyl derivative **9** was prepared according to the procedure of Zhdanov *et al.*[11] Deacetylation of **9** gave 2-(β-D-glucopyranosyl)-

1-propene (**10**) Since the acetates **15** and **16**, obtained by hydrogenation of **1** and **9**, do not contain a double bond and exhibit almost identical molecular rotation (−50° and −52°, respectively), a structure similar to that of **1** was ascribed to **9**

In contrast to *O*-allyl glycosides, which are completely converted into epoxides within 3 hours by a 2–4 times excess of peracid[3 12], **9** required 24 hours, and **1** 4 days, provided that a 10 times excess of peracid was present for the oxidation of the latter compound

The free epoxides **5**, **6**, **13**, and **14** are unstable, being decomposed even when rapidly treated with Dowex-50(H^+) in a methanol solution Hence, the alkaline solutions resulting from the deacetylation were neutralized by carbone dioxide A gradual decomposition of these compounds was also observed when they were stored as well dried syrups at −10° The acetates **7**, **8**, **11**, and **12** are sufficiently stable

A preliminary study of epoxides **5**, **6**, **13**, and **14** with sweet-almond β-D-glucosidase showed that the enzyme was irreversibly inactivated A more detailed account of these results will be published later

EXPERIMENTAL

General methods — T l c was performed on Silica Gel LS 5–40 μm (La Chema, ČSSR) containing 10% of gypsum with 4 1 ether–light petroleum (*A*), for acetates, and with 7 3 chloroform–methanol (*B*), for hydroxyl-containing derivatives, the spots were revealed by conc sulfuric acid at 100–120° Column chromatography was performed on Silica Gel LS 40–100 μm (for separation of the **7**, **8** and **11**, **12** mixtures) and LS 100–150 μm (in other cases), the ratio of weight of substance to weight of adsorbent being 1·100 and the ratio of diameter to length of columns 1 25 Paper chromatography was performed on Whatman No 1 paper with 14 3 2 acetone–butyl alcohol–water, and detection with alkaline silver nitrate[13] Solutions were evaporated *in vacuo* at a bath temperature below 45° Melting points were determined with a Boetius apparatus and are corrected Optical rotations were determined with a Perkin–Elmer Model 141 M polarimeter I r. spectra were recorded, for potassium bromide discs, with a Perkin–Elmer Model 257 spectrophotometer P m r spectra were recorded at 100 MHz with a Varian XL-100 spectrometer, for solutions in chloroform-*d* with tetramethylsilane as internal standard (abbreviations p, proton, s, singlet; t, triplet, q, quartet, and m, multiplet) M s were recorded with a LKB Model 9000 spectrometer at 70 eV and an ion-source temperature of 270°.

(*2,3,4,6-Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*ethene* (**1**) — A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (15 g) in dry tetrahydrofuran (150 ml) was added to a stirred solution of vinyl magnesium bromide [prepared from vinyl bromide (60 ml) and magnesium (14 g)] in dry tetrahydrofuran (250 ml) for 2 h at 40° The solution was stirred for 5 h at 60°, cooled, and treated with water (100 ml) and 30% hydrochloric acid (200 ml) The aqueous layer was separated, extracted with ether (3 × 100 ml), and evaporated to dryness, the residue was dried *in vacuo* for 6 h at 50°. The residue was acetylated by stirring with acetic anhydride (300 ml) and

sodium acetate (15 g) for 3 h at 100°, poured onto ice, extracted with chloroform (4 × 200 ml), washed with a sodium hydrogen carbonate solution (3 × 100 ml) and with water, and evaporated The residue was chromatographed on silica gel with an increasing concentration of ether in light petroleum (1 5 l) The major component of the mixture was isolated as a syrup that crystallized from light petroleum–ether to give 4 5 g (34%) of **1**, m p 102 5–103° (lit.[8] m p 88–89°); $[\alpha]_D^{20}$ +14° (*c* 1, chloroform), t l c R_F 0.49, i r data ν_{max}^{KBr} 1650 (C=C), 1750 (OAc), 3000, 3080 cm^{-1} (C–H, CH_2 in CH=CH_2), n m r data δ 5 6–6 0 (m, –CH=), 4 9–5 4 (m, CH_2=), 4 2 (m, 2H-6′), 2 12 (s, 3 p, OAc), 2 06 (s, 3 p, OAc), and 2 03 (s, 6 p, OAc), m s *m/e* 358 ($M^{\ddagger}$) and 359 ($M^+ + 1$)

Anal Calc for $C_{16}H_{22}O_9$ C, 53 6, H, 6 2 Found· C, 53 8, H, 6 1

(*2,3,4,6-Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*methyl acetate* (**4**) — A 1% aqueous solution of osmic anhydride (6 ml) was added to a solution of **1** (270 mg) in *p*-dioxane (10 ml) and then sodium periodate (400 mg) was introduced within 15 min The suspension was stirred for 1 5 h and extracted with chloroform (3 × 10 ml) The extracts were evaporated and the residue (**3**, R_F 0 2, ether) was dissolved in ethanol (10 ml) The solution was stirred with sodium borohydride (60 mg) for 24 h, filtered, treated with Amberlite MB-6, and evaporated to dryness The residue was acetylated with acetic anhydride (0 5 ml) in pyridine (2 ml), chromatographed on silica gel, as described for **1**, and crystallized from 2-propanol to yield **4** (75 mg), m p 95 5–96 5°, $[\alpha]_D^{20}$ 0±0 2° (*c* 1, chloroform), t l c R_F 0 3, m s *m/e* 405 ($M^+ + 1$)

Anal. Calc for $C_{17}H_{24}O_{11}$· C, 50 5; H, 6 0 Found C, 50 7, H, 6 1

Compound **4** prepared by an alternative synthesis[9] had a double m p., 87–88° and 96–97° (lit [9] m p 89°), mixed m p 96–97° The i r spectra of both samples are identical

β-D-*Glucopyranosylethene* (**2**) — A 0 1M sodium methoxide solution was added to a solution of **1** (2 1 g) in dry methanol (50 ml) to pH 8 After 2 h, the solution was treated with Dowex-50 (H^+) resin and evaporated to give syrupy **2** (1 05 g, 92%), $[\alpha]_D^{20}$ +30° (*c* 1, methanol), t l c R_F 0 5, p c R_{Glc} 2 0, i r data ν_{max}^{KBr} 1650 (C=C) and 3200–3500 cm^{-1} (OH)

Anal Calc for $C_8H_{14}O_5$ C, 50 5, H, 7 4 Found C, 50 2, H, 7 7

(*2,3,4,6-Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*ethane* (**15**) — A solution of **1** (90 mg) in ethanol (20 ml) was hydrogenolyzed in the presence of 20% palladium-on-carbon (100 mg) for 18 h The reaction mixture was filtered and evaporated The residue was chromatographed on silica gel, as described for **1**, to give syrupy **15** (80 mg, 89%), $[\alpha]_D^{20}$ −14° (*c* 1, chloroform), t l c R_F 0 55, i r data ν_{max}^{KBr} 1750 cm^{-1} (OAc), n m r data δ 4 2 (m, 2 H-6′), 3 5–3.8 (m, H-5′), 2 07 (s, 3 p, OAc), 2 02 (s, 3 p, OAc), 2 01 (s, 3 p, OAc), 1 99 (s, 3 p, OAc), 1 5 (m, Et CH_2), and 0 96 (t, *J* 7 Hz, CH_3), m s · *m/e* 361 ($M^{\ddagger} + 1$)

Anal Calc for $C_{16}H_{24}O_9$ C, 53 3, H, 6 7 Found C, 53 1, H, 6 6

Diastereomeric epoxy-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*ethanes* (**7** *and* **8**) — A suspension of peroxyphthalic acid (80% of active oxygen, 1 14 g, 5 mmoles) in a solution of **1** (358 mg, 1 mmole) in dichloromethane (5 ml) was heated in a sealed

flask at 43° for 2 days, then peroxyphthalic acid (1 14 g) was once again added, and the suspension heated for another 2 days. The mixture was kept overnight at 0°, and then the precipitate was filtered off and washed with cold dichloromethane (2 × 10 ml) The filtrates were treated with a cold 0 1M potassium hydrogen carbonate solution (2 × 10 ml) and evaporated to give a residue, which was chromatographed on silica gel with Solvent *A* The more mobile epoxide **7** was isolated as syrup (108 mg, 29%) and crystallized from ether–light petroleum, m p 102–104°, $[\alpha]_D^{20}$ +3° (*c* 1, chloroform), t l c R_F 0 32, i r data ν_{max}^{KBr} 1755 (OAc), 3000, 3030, and 3060 cm^{-1} (C–H in epoxide ring), n m r. data δ 4 27, 4 08 (2 q, 2 H-6′), 3 5–3 7 (m, H-5′), 2 9–3 1, 2 6–2 8 (2 m, $\ulcorner O \urcorner$ CH–CH_2), 2 05 (s, 6 p, OAc), and 1.99 (s, 6 p, OAc), m s *m/e* 375 (M^+ +1), *m/e* 417 (M^+ +43)

Anal Calc for $C_{16}H_{22}O_{10}$ C, 51 3, H, 5 9 Found. C, 51 1, H, 5 9

Diastereomeric epoxide **8** was isolated in a yield of 217 mg (58%), m p 143–145° after recrystallization from ether–light petroleum, $[\alpha]_D^{20}$ +12° (*c* 1, chloroform), t l c R_F 0.24, i r data ν_{max}^{KBr} 1755 (OAc), 3005, 3020, and 3060 cm^{-1} (C–H in epoxide ring), n m r data δ 4 2 (m, 2 H-6′), 3 6–3 8 (m, H-5′), 3 0–3 2, 2 8–2 9, 2 5–2 7 (3 m, $\ulcorner O \urcorner$ CH–CH_2), 2 11 (s, 3 p, OAc), 2 05 (s, 6 p, OAc), and 2 03 (s, 3 p, OAc), m s *m/e* 375 (M^+ + 1) and 417 (M^+ +43)

Anal. Calc for $C_{16}H_{22}O_{10}$ C, 51 3, H, 5 9 Found C, 51 3, H, 6 0

Diastereomeric epoxy-(β-D-glucopyranosyl)ethanes (**5** *and* **6**) — A solution of 0 1M sodium methoxide was added to a solution of **7** (94 mg) in dry methanol (25 ml) to pH 8 The solution was kept overnight at 5°, treated with carbon dioxide, evaporated to 10 ml, passed through a layer of silica gel (3 × 5 cm) with Solvent *B* (150 ml), and evaporated to dryness to give syrupy **5** (46 mg, 90%), $[\alpha]_D^{20}$ −14° (*c* 1, methanol), t l c R_F 0 45, p c R_{Glc} 1 65, i r data ν_{max}^{KBr} 1265 (C–O in epoxide ring), 1650, and 3200–3500 cm^{-1} (OH)

Anal Calc for $C_8H_{14}O_6$ C, 46 6, H, 6 8 Found C, 46 5, H, 6 9

Compound **8** was deacetylated, as described for **7**, to give syrupy **6** in a yield of 94%, $[\alpha]_D^{20}$ −6° (*c* 1, methanol), t l c R_F 0.45, p c R_{Glc} 1 65, i r data ν_{max}^{KBr} 1265 (C–O in epoxide ring), 1645, and 3200–3500 cm^{-1} (OH)

Anal Calc for $C_8H_{14}O_6 \cdot$ C, 46 6, H, 6 8 Found C, 46 4, H, 7 0

Both **5** and **6** gave an intense positive reaction indicating the presence of an epoxide group[6], when tested with a sodium thiosulfate solution.

2-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1-propene (**9**) — A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (15 g) in dry ether (100 ml) was added to a stirred solution of Grignard reagent [prepared from allyl bromide (45 ml) and magnesium (12 g) in dry ether (250 ml)] for 2 h under reflux The solution was heated and stirred for an additional 5 h under reflux and treated, acetylated, chromatographed, and crystallized, as described for **1**, to give **9** in a yield of 5 0 g (37%), m p 78–78 5°, $[\alpha]_D^{20}$ −8° (*c* 1, chloroform), t l c R_F 0 51, i r. data ν_{max}^{KBr} 1645 (C=C), 1750 (OAc), 3020, and 3080 cm^{-1} (C–H, CH_2 in $CH{=}CH_2$), n m r data· δ 5 5–6 0

(m, –CH=), 4 7–5 4 (m, CH_2=), 4 28, 4.08 (2 q, 2 H-6′), 2 09 (s, 3 p, OAc), 2 03 (s, 6 p, OAc), and 2 01 (s, 3 p, OAc), m s *m/e* 373 ($M^+ + 1$) (*cf.* Ref 11)

Anal Calc for $C_{17}H_{24}O_9$ C, 54 8; H, 6 5. Found· C, 54 9, H, 6 6

2-(β-D-Glucopyranosyl)-1-propene (**10**) — Syrupy **10** was obtained from **9**, as described for **2**, in a yield of 92%, $[\alpha]_D^{20}$ −6° (*c* 1, methanol), t l c R_F 0 55; p c R_{Glc} 2 5; i r data ν_{max}^{KBr} 920 (CH_2 in CH=CH_2, deform), 3010, 3030 (C–H, CH_2 in CH=CH_2), and 3200–3500 cm^{-1} (OH)

Anal Calc for $C_9H_{16}O_5$ C, 52 9, H, 7 9. Found C, 52 8, H, 7 9

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)propane (**16**) — Hydrogenolysis of **9**, similar to that of **1**, gave syrupy **16** in a yield of 95%, $[\alpha]_D^{20}$ −14° (*c* 1, chloroform), t l c· R_F 0 56, i r data ν_{max}^{KBr} 1750 cm^{-1} (OAc), n m r data δ 4 26, 4 07 (2 q, 2 H-6′), 3 5–3 7 (m, H-5′), 2 06 (s, 3 p, OAc), 2 02 (s, 3 p, OAc), 2 00 (s, 3 p, OAc), 1 98 (s, 3 p, OAc), 1 4, and 0 9 (2 m, $CH_2CH_2CH_3$), m s *m/e* 375 ($M^+ + 1$)

Anal Calc for $C_{17}H_{26}O_9$. C, 54 5, H, 7 0 Found C, 54 4, H, 7.1.

Diastereomeric 1,2-epoxy-3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)propanes (**11** *and* **12**) — A suspension of peroxyphthalic acid (910 mg) in a solution of **9** (372 mg) in dichloromethane (5 ml) was heated for 24 h at 43°, treated, chromatographed, and crystallized, as described for **1**, to give **11** and **12** The more mobile epoxide **11** was isolated in a yield of 233 mg (60%), m p 116–118°, $[\alpha]_D^{20}$ −22° (*c* 1, chloroform), t l c R_F 0 27, i r data ν_{max}^{KBr} 1755 (OAc), 3030, 3060 cm^{-1} (C–H in epoxide ring), n m r. data. δ 4 31, 4 12 (2 q, 2 H-6′), 3 1–3 3, 2 8–2 9, 2 5–2 6

(3 m, CH–CH_2), 2 10 (s, 3 p, OAc), 2 05 (s, 6 p, OAc), and 2 02 (s, 3 p, OAc), m s *m/e* 389 ($M^+ + 1$) and 431 ($M^+ + 43$)

Anal. Calc for $C_{17}H_{24}O_{10}$: C, 52 6, H, 6 2 Found C, 52 6; H, 6 3

Diastereomeric epoxide **12** was isolated in a yield of 58 mg (15%), m p 113–115°, $[\alpha]_D^{20}$ −10° (*c* 1, chloroform), t l c . R_F 0 23, i r data ν_{max}^{KBr} 1755 (OAc), 3010, 3030, and 3060 cm^{-1} (C–H in epoxide ring), n m r data δ 4 2 (m, 2 H-6′), 3 0–3 2,

2 7–2 8, 2 4–2 6 (3 m, CH–CH_2), 2 06 (s, 3 p, OAc), 2 00 (s, 6 p, OAc), and 1 98 (s, 3 p, OAc), m s *m/e* 389 ($M^+ + 1$) and 431 ($M^+ + 43$)

Anal Calc for $C_{17}H_{24}O_{10}$ C, 52 6, H, 6 2 Found C, 52 4, H, 6 3

Diastereomeric 1,2-epoxy-3-(β-D-glucopyranosyl)propanes (**13** *and* **14**) — Syrupy **13** and **14** were obtained from **11** and **12**, respectively, as described for **5** and **6**

The epoxide **13** was isolated in a yield of 90%, $[\alpha]_D^{20}$ −14° (*c* 1, methanol), t l c R_F 0 47, p c· R_{Glc} 1 8, i r data ν_{max}^{KBr} 1265 (C–O in epoxide ring), 1645, and 3200–3500 cm^{-1} (OH)

Anal Calc. for $C_9H_{16}O_6$ C, 49.1, H, 7.3 Found C, 49.0; H, 7.5.

The epoxide **14** was isolated in a yield of 94%, $[\alpha]_D^{20}$ −2° (*c* 1, methanol); t l c R_F 0 47; p c R_{Glc} 1.8; i r data ν_{max}^{KBr} 1265 (C–O in epoxide ring), 1645, and 3200–3500 cm^{-1} (OH)

Anal Calc. for $C_9H_{16}O_6$ C, 49 1, H, 7 3. Found C, 48 8; H, 7 4.

Both **13** and **14** like **5** and **6** show a positive test[6] for an epoxide group.

REFERENCES

1 B R BAKER, *Design of Active-Site-Directed Irreversible Enzyme Inhibitors*, Wiley, New York, 1967
2 G LEGLER AND S N HASNAIN, *Z Physiol Chem*, 351 (1970) 25, G LEGLER, *ibid*, 349 (1968) 767, G LEGLER, *Biochim Biophys Acta*, 151 (1968) 728
3 E W THOMAS, *Carbohyd Res*, 13 (1970) 225
4 J MOULT, Y ESHDAT, AND N SHARON, *J Mol Biol*, 75 (1973) 1, and literature cited therein
5 F NAIDER, Z BOHAK, AND J YARIV, *Biochemistry*, 11 (1972) 3202, J YARIV, K J WILSON, J HILDESHEIM, AND S BLUMBERG, *FEBS Lett*, 15 (1971) 24
6 W C J ROSS, *J Chem Soc*, (1950) 2257
7 G LEGLER AND H GILLES, *Z Physiol Chem*, 351 (1970) 741
8 YU A ZHDANOV, G A KOROLCHENKO, L A KUBASSKAYA, AND R M KRIVORUCHKO, *Dokl Akad Nauk SSSR*, 129 (1959) 1049
9 B COXON AND H G FLETCHER, JR, *J Amer Chem Soc*, 85 (1963) 2637
10 S D SHIYAN, M L SHULMAN, AND A YA KHORLIN, *Izv Akad Nauk SSSR, Ser Khim*, (1973) 2386
11 YU A ZHDANOV, L I SHCHERBAKOVA, AND T N EGOROVA, *Dokl Akad Nauk SSSR*, 83 (1952) 403
12 E M BESSELL AND J H WESTWOOD, *Carbohyd Res*, 25 (1972) 11
13 W E TREVELYAN, D P PROCTER, AND J S HARRISON, *Nature*, 166 (1950) 444