Tingenone and Hydroxytingenone, Triterpenoid Quinone Methides from *Euonymus tingens*

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Spectroscopic and crystallographic studies have established that the pigment tingenone is a triterpenoid quinone methide closely related to pristimerin. A second pigment isolated from *E. tingens* is 20-hydroxytingenone.

It has been suggested [1] that tingenone, an orange pigment found in the bark of *Euonymus tingens* Wall., is a naphthoquinone. If so the intense visible absorption [$\lambda_{max.}$ 425 nm (log ε 4·11) †] is highly anomalous but would be consistent with certain quinone methide structures such as pristimerin (I; R = Me) [2] [423 nm (log ε 4·10)] and fuerstione (II) [3] [445 nm (log ε 4·09)]. Pristimerin (I; R = Me), and also celastrol (I; R = H),[4] occur in the bark and roots of several plants of the Celastraceae family to which *Euonymus* belongs. Direct comparison of the electronic spectra of tingenone and pristimerin showed that they were identical. Furthermore, the two n.m.r. spectra are identical in the low-field region; H-1, in pristimerin, resonates at τ 3·47, and H-6 and H-7 appear as doublets (J 7 Hz) centred at 2·99 and 3·65, respectively, the former partially obscured by the hydroxy-singlet at τ 3·02. In both spectra a singlet (3H) at τ 7·79 can be attributed to a quinonoid methyl group. When the hydroxy-signal at τ 3·02 was removed from the tingenone spectrum with D_2O the long-range coupling between H-1 and H-6 could be observed ($J_{1,6}$ *ca.* 1 Hz).[5] Both tingenone and pristimerin, in chloroform, give a red colour with sodium hydroxide, the colour remaining in the chloroform layer. Thus the hydroxyquinone methide chromophore of (I) is common to both pigments.

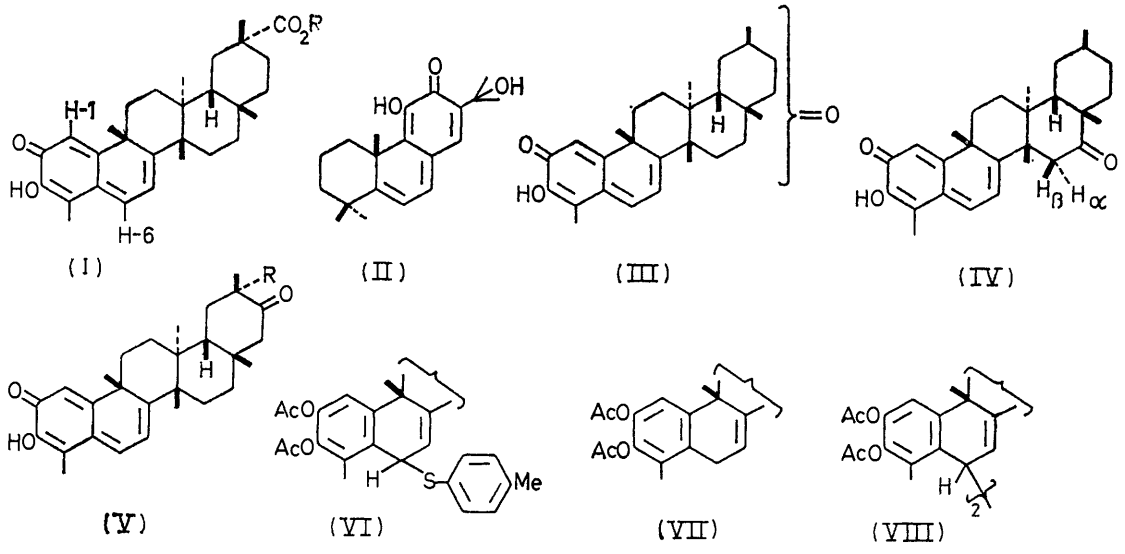
A close relationship is also suggested by the i.r. spectra

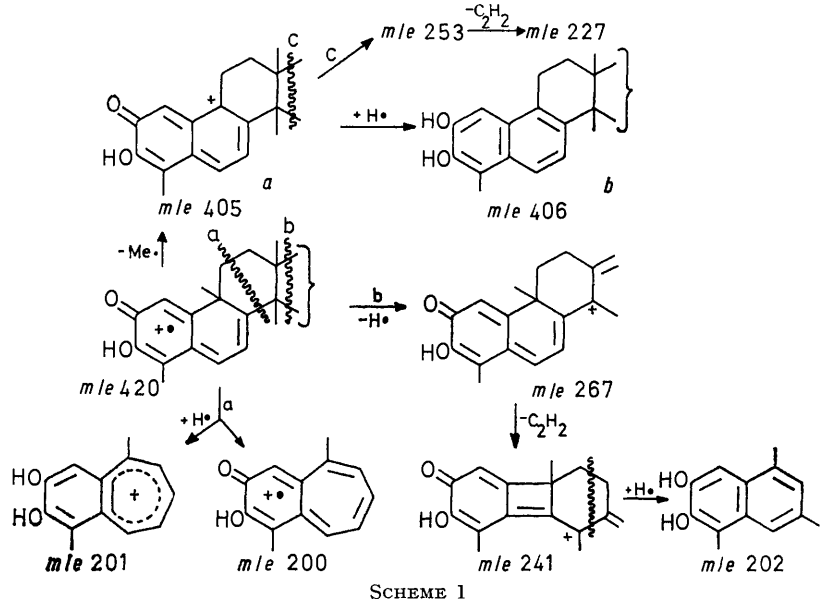
† Not log ε 5·02, which was based on an incorrect molecular formula.[1]

[1] V. Krishnamoorthy, J. D. Ramanathan, and T. R. Seshadri, *Tetrahedron Letters*, 1962, 1047.
[2] P. K. Grant and A. W. Johnson, *J. Chem. Soc.*, 1957, 4079; A. W. Johnson, P. F. Juby, T. J. King, and S. W. Tam, *ibid.*, 1963, 2884; R. Harada, H. Kakisawa, S. Kobayashi, M. Musya, K. Nakanishi, and Y. Takahashi, *Tetrahedron Letters*, 1962, 603; P. J. Ham and D. A. Whiting, *J.C.S. Perkin I*, 1972, 330.
[3] D. Karanatsios, J. S. Scarpa, and C. H. Eugster, *Helv. Chim. Acta*, 1966, **49**, 1151.
[4] K. Nakanishi, H. Kakisawa, and Y. Hirata, *Bull. Chem. Soc. Japan*, 1956, **29**, 7.
[5] K. Nakanishi, Y. Takahashi, and H. Budzikiewicz, *J. Org. Chem.*, 1965, **30**, 1729.

of these compounds, which are very similar except for the region near 1700 cm^{-1} where the ester carbonyl band of pristimerin at 1730 cm^{-1} ($CHCl_3$) is replaced in the tingenone spectrum by ketonic carbonyl absorption at 1710 cm^{-1}. The resemblance is further confirmed by comparison of the two mass spectra, which are virtually identical at *m/e* values below 300; significant peaks, *inter alia*, at *m/e* 267, 253, 241, 227, 202, 201, and 200 suggest that major fragmentations occur at the C/D ring junction with hydrogen transfer (*cf.* friedelane derivatives [6]), as indicated for tingenone in Scheme 1. Peaks at $M - 14$ in the spectra of both pigments probably correspond to the dihydroxynaphthalene ions *b*, formed from *a* by reaction with a hydrogen donor (possibly *a*). The molecular formulae of all these ions were confirmed by accurate mass measurement, but unfortunately the tingenone spectrum is devoid of metastable peaks (except for that corresponding to the fragmentation M — Me). It can be concluded from these fragmentations that rings A, B, and C are the same in both tingenone and pristimerin.

The molecular ion of tingenone appears at *m/e* 420 ($C_{28}H_{36}O_3$) and there is also a peak at *m/e* 436 (*ca.* 20%), the relative intensity of which varies with the operating conditions. This is evidently due to a hydroxylic impurity (not detected by t.l.c.) and may partly account for the low % carbon values obtained on combustion. As the molecular formula of pristimerin is $C_{30}H_{38}O_4$, the two compounds have the same number of double bond equivalents, so tingenone must contain a cyclic ketonic group instead of the methyl ester function in pristimerin.





SCHEME 1

[6] J. L. Courtney and J. S. Shannon, *Tetrahedron Letters*, 1963, 13.

On the basis of both spectroscopic and phytochemical evidence it seems likely that these pigments have the same carbon skeleton, but whereas the n.m.r. spectrum of pristimerin clearly displays singlets from five aliphatic tertiary methyl groups (τ 8·56, 8·73, 8·81, 8·89, and 9·45) that of tingenone is less well-defined, with singlets (each 3H) at τ 8·50 and 8·65, and a broad signal (9H) centred at τ 9·01. However, in $CDCl_3$–C_6D_6 (1 : 1) solution the latter separates into two singlets (each 3H) at τ 9·13 and 9·29, and a doublet centred at τ 9·02. Structure (III) can thus be suggested for tingenone, the remaining problem being the location of the ketonic group in ring D or E.

In this connection there is a significant doublet (1H) in the n.m.r. spectrum at τ 7·09 (J 15 Hz); spin decoupling showed that this exhibited coupling to a proton resonating at τ 8·12 in the methylene–methine 'hump.' The latter signal is normal for a proton α to a carbonyl group but the former is outside the usual range, which rarely extends below τ 7·2.[7] Examination of Dreiding models of the four likely isomers of structure (III) revealed that if the ketonic group is placed at C-16 [structure (IV)], then the α-proton at C-15 (H_α) lies in the planes of both the 7,8-double bond and the ketonic double bond. It was considered that deshielding of the α-proton by both these groups would account for its chemical shift, and the C-16 ketone structure was tentatively adopted.[8] As chemical proof of this structure was difficult, confirmation was then sought by crystallographic analysis, which, however, showed that the structure (IV) was incorrect.

Tingenone does not readily form single crystals suitable for X-ray analysis but one sample consisted of relatively massive conglomerates, a chip from one of which appeared to be a single crystal as judged by oscillation and Weissenberg photographs. From these photographs and subsequent measurements on a Hilger–Watt four-circle diffractometer (Cu-K_α radiation) the crystal was shown to belong to space group $P2_12_12$ (a = 13·859, b = 15·297, c = 11·096 Å), with the expected four molecules to the unit cell. The intensities of 2407 observable reflections were measured out to a θ angle of 78°.

The structure was determined using MULTAN. The four E-maps with the highest figures of merit showed very large peaks at the origin and were clearly anomalous, but the 31 highest peaks in the E-map with the fifth highest figure of merit formed a chemically sensible and recognisable pattern corresponding to a compound with a pristimerin skeleton lacking the methoxycarbonyl group at C-20 and with an extra substituent at C-21, which, in view of the geometry at this centre, was apparently a carbonyl oxygen atom.

Refinement of this structure is not yet complete but it is apparent that the final R-value is unlikely to be very small, probably about 10%. Bond lengths and bond angles are, however, in reasonable agreement with normally accepted values for such a compound. Difference maps plotted at several stages of the refinement showed only one major peak which was within bonding distance of C-22 and appears to arise from the hydroxy-containing impurity referred to earlier. No evidence was given at any time for the presence of a substituent at C-16. Refinement of the tingenone structure with the occupation number of the additional oxygen being varied suggested that the particular crystal being investigated contained about 25% of this hydroxy-compound. We believe that it is the presence of this substantial amount of impurity which is responsible for the relatively unsatisfactory refinement of the structure. Full details of the X-ray investigation will be published in a specialist journal. As the c.d. curves for tingenone and pristimerin are very similar, the two pigments must have the same absolute configuration. One of the protons at C-22 must be responsible for the doublet at τ 7·09 in the n.m.r. spectrum of tingenone.

It has been shown [9] that tingenone is identical with maitenin, a pigment isolated from *Maytenus* spp. and other Celastraceae.

We found it difficult to prepare simple derivatives of tingenone in a pure state but two products were obtained. Addition of toluene-*p*-thiol, followed by acetylation, gave a diacetate to which we assign structure (VI). It had the expected spectroscopic properties; in particular the n.m.r. spectrum showed a doublet (1H) at τ 4·22 coupled to another (1H) at τ 5·20, assigned to H-7 and H-6, respectively. The second derivative was the 'leucodiacetate' prepared by reduction with zinc and acetic anhydride. This was not the expected product (VII): the n.m.r. spectrum showed that the ratio of H-1, H-6, and H-7 signals was 1 : 1 : 1, and not 1 : 2 : 1 as required by structure (VII). As the molecular weight (osmometric) of this compound was found to be 1005 we regard it as the dimer (VIII) (M 1010); this structure is consistent with all the spectroscopic evidence. There is no molecular ion in the mass spectrum and the peaks at highest mass form a cluster at m/e 504, 505, and 506, the relative intensities of which vary with the operating conditions. This suggests that the molecular ion readily cleaves at the 6,6′-bond to form the ion c, which disproportionates giving d and e (Scheme 2). The more intense peak at m/e 490 is probably the naphthalene radical-ion f. The tolylthio-derivative (VI) fragments in similar fashion. The molecular ion does not appear in the mass spectrum; there is a minute peak at M − 1 and another at M − 16 (1%) attributable to the ion g, but loss of the tolylthio-group is much more important. This affords ion c at m/e 505 (30%) (followed by the sequence 490 ⟶ 448 ⟶ 406 as in Scheme 2) and the tolylthio-ion at m/e 123 (90%), the base peak being at m/e 124.

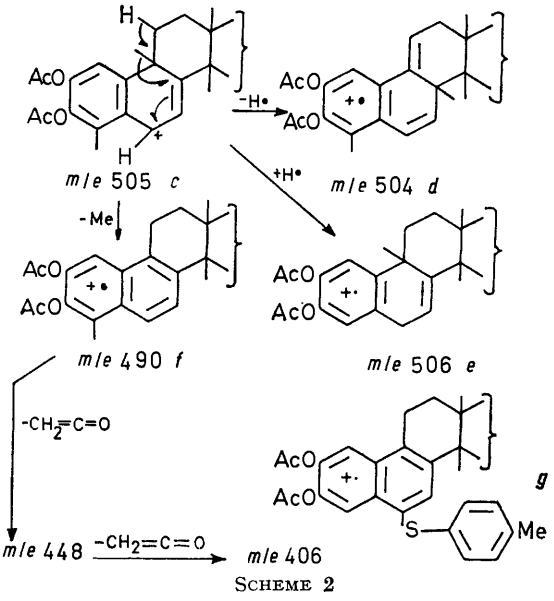
The n.m.r. spectrum of the dimeric acetate (VIII) in the low-field region is in good agreement with the

[7] N. S. Bhacca and D. H. Williams, 'Applications of NMR Spectroscopy in Organic Chemistry,' Holden-Day, San Francisco, 1964, p. 63.

[8] V. Krishnamoorthy, T. R. Seshadri, R. H. Thomson, and M. Moir, Abstracts IUPAC 8th International Symposium on the Chemistry of Natural Products, New Delhi, 1972, p. 165.

[9] F. Delle Monache, G. B. Marini-Bettòlo, P. M. Brown, M. Moir, and R. H. Thomson, *Gazzetta*, 1973, in the press.

spectra [5] of the acetates obtained by reduction of pristimerin (I; R = Me) and celastrol (I; R = H) with zinc and acetic anhydride, and there is little doubt that these anomalous compounds are also 6,6′-dimers. Although the formation of dimers during reductive acetylation appears to be new this is not unknown in other zinc reductions, *e.g.* the Clemmensen [10] reduction of ketones occasionally yields pinacols.



SCHEME 2

The tingenone mother liquors contain several other pigments, one of which has been obtained pure by extensive chromatography. This compound, $C_{28}H_{36}O_4$, has the same chromophore and ketonic group as tingenone, and possesses an additional oxygen atom in the form of a tertiary hydroxy-group. This follows from the presence of two hydroxy-bands in the i.r. spectrum, an additional singlet in the n.m.r. spectrum at τ 6·65 (exchangeable with D_2O) but no >C*H*·OH signals. As *all* the methyl signals are singlets (two being coincident) the new pigment must be 20-hydroxytingenone (V; R = OH). The mass spectrum is very similar to that of tingenone, apart from intensity differences, and the general forms of the c.d. curves are also similar. Presumably both pigments have the same stereochemistry throughout but this cannot be deduced from the c.d. curve, which is probably dominated by the A and B ring chromophore. In the n.m.r. spectrum of this compound the doublet arising from one of the 22-protons α to the ketonic group has shifted to still lower field (τ 7·01).

EXPERIMENTAL

Tingenone (V; R = H).—Ground bark of *E. tingens* (1 kg) was extracted (Soxhlet) with light petroleum (4 l). The solid which separated on cooling was crystallised repeatedly from benzene–light petroleum and ethyl acetate to give *tingenone* (1 g) as orange crystals which were homogeneous in several t.l.c. systems. The m.p. varied with the solvent used [9,11] and the manner of drying; m.p.s within the range 140—240° were recorded but most samples melted near 180—190°; a sample crystallised from ethyl acetate, then dissolved in cyclohexane, recovered by evaporation *in vacuo*, and dried overnight at 75° and 0·05 mmHg had m.p. 189—192° (Found: C, 79·6; H, 8·8%; M^+, 420·2674. $C_{28}H_{36}O_3$ requires C, 80·0; H, 8·6%; *M*, 420·2664), $\lambda_{max.}$ (EtOH) 252sh and 425 nm (log ε 3·95 and 4·11), c.d. $\lambda_{max.}$ (MeOH) 258 (Δε − 8·81), 263 (−9·20), 372 (+7·90), and 451 nm (−4·31) [*cf.* pristimerin, 258 (−7·10), 264 (−8·01), 372 (+7·10), and 445 (−3·43)], $\nu_{max.}$ ($CHCl_3$) 3395, 1710, 1652, and 1598 cm^{-1}, τ (100 MHz; $CDCl_3$) 2·99 (1H, q, *J* 7 and 1 Hz, H-6), 3·02 (1H, s, OH, exchangeable with D_2O), 3·47 (1H, d, *J* 1 Hz, H-1), 3·65 (1H, d, *J* 7 Hz, H-7), 7·09 (1H, d, *J* 15 Hz), 7·79 (3H, s, quinone CH_3), 8·50 and 8·65 (each 3H, s, CH_3), and 9·01 (9H, 3 × CH_3) [in C_6D_6–$CDCl_3$ (1 : 1) the CH_3 signals are singlets at τ 8·69, 8·90, 9·12, 9·29, and a doublet at 9·02 (*J* 6 Hz)], *m/e* 436 (22%), 421 (35), 420 (100), 407 (5), 406 (13), 405 (10), 267 (11), 253 (24), 241 (59), 227 (20), 219 (22), 215 (11), 202 (92), 201 (93), 200 (67), 187 (16), 163 (23), 135 (22), 121 (21), 109 (29), 107 (20), 95 (35), and 91 (28).

Reductive Acetylation.—A mixture of tingenone (50 mg), zinc dust (50 mg), and sodium acetate (25 mg) in acetic anhydride (2 ml) was heated under reflux for 30 min, cooled and poured on ice. The product was extracted with chloroform, and crystallised from methanol–chloroform to give the *dimer* (VIII) as prisms, m.p. 226—228° [Found: C, 75·3; H, 8·3%; *M* (osmometric), 1005. $C_{64}H_{82}O_{10}$ requires C, 76·0; H, 8·2%; *M*, 1010], $\nu_{max.}$ (KBr) 1777, 1712, and 1217 cm^{-1}, τ ($CDCl_3$) 2·92 (2H, s, ArH), 4·07 (2H, m, −CH=), 6·32 (2H, m, ArC*H*–CH=), 7·08 (2H, d, *J* 14 Hz, >CH·CO), 7·75 (12H, s, OAc), 8·11 (6H, s, $ArCH_3$), 8·57 and 8·79 (each 6H, s, CH_3), and 9·01 (18H, ' d,' 3 × CH_3), *m/e* 506 (7·5%), 505 (2), 504 (3), 491 (12), 490 (29), 449 (28), 448 (91), 407 (29), 406 (100), 391 (10), 269 (30), 253 (20), 239 (40), 227 (94), 201 (97), 188 (18), 109 (23), 95 (39), 81 (32), 69 (24), 67 (30), 55 (53), and 43 (61).

*Addition of Toluene-*p*-thiol.*—Toluene-*p*-thiol (30 mg) in methanol (5 ml) was added to tingenone (100 mg) suspended in the same solvent (10 ml). The solution rapidly became pale yellow. After 30 min the solvent was removed *in vacuo* and the residual gum was dissolved in acetic anhydride (2 ml) containing pyridine (0·3 ml), and left overnight. The mixture was poured on ice and extracted with ethyl acetate; the extract was washed with water, aqueous sodium hydrogen carbonate, dilute hydrochloric acid, and water, dried ($MgSO_4$), and evaporated. The residue was purified by p.l.c. (3 times) on silica gel in chloroform. The *product* (VI) separated from aqueous methanol as a white solid, m.p. 123—125° (Found: C, 74·8; H, 7·9; S, 5·1. $C_{39}H_{48}O_5S$ requires C, 74·5; H, 7·7; S, 5·1%), $\lambda_{max.}$ (MeOH) 215 and 236sh nm (log ε 4·34 and 4·05), $\nu_{max.}$ (KBr) 1774, 1710, and 1209 cm^{-1}, τ ($CDCl_3$) 2·62 and 2·87 (each 1H, d, *J* 8 Hz, ArH), 2·94 (1H, s, ArH), 4·22 (1H, d, *J* 5 Hz, −CH=), 5·20 (1H, d, *J* 5 Hz, ArC*H*–CH=), 7·12 (1H, d, *J* 15 Hz, >CH·CO), 7·66 (9H, s, CH_3·CO_2 and $ArCH_3$), 7·72 (3H, s, $ArCH_3$), 8·43 (3H, s, CH_3), 8·67 (3H, s, CH_3), and 9·03 (9H, m, 3 × CH_3), *m/e* 627 (0·1%), 612 (1), 506 (12), 505 (30), 490 (25), 448 (56), 406 (41), 285 (34), 246 (78), 243 (43), 228 (27), 202 (41), 201 (64), 124 (100), 123 (90), 91 (91), 79 (24), 77 (24), and 43 (54).

20-Hydroxytingenone (V; R = OH).—The tingenone mother liquors were evaporated and chromatographed on a

[10] E. L. Martin, *Org. Reactions*, 1942, **1**, 155.
[11] O. Gonçalves de Lima, J. Sidney de Barros Coêlho, E. Weigert, I. L. d'Albuquerque, Dardano de Andrade Lima, and M. Alves de Moraes e Souza, *Rev. Inst. Antibiot. Recife*, 1971, **11**, 35.

column of silica gel in benzene–ethyl acetate (10 : 4) to give a fraction containing two pigments with R_F (t.l.c.) lower than that of tingenone. One of these was isolated by repeated p.l.c. on silica gel [first in benzene–ethyl acetate (10 : 4), then, after drying, in benzene–ethyl acetate (1 : 1)]. 20-*Hydroxytingenone* separated from acetone as bright red crystals, m.p. 207—208·5° (Found: C, 76·8; H, 8·2%; M^+, 436·2585. $C_{28}H_{36}O_4$ requires C, 77·0; H, 8·3%; M, 436·2613), $\lambda_{max.}$ (EtOH) 256sh and 426 nm (log ε 3·97 and 4·05), c.d. $\lambda_{max.}$ (MeOH) 222 ($\Delta\varepsilon$ −4·32), 264 (−10·40), 301 (+2·59), 377 (+9·90), and 446—456 (−3·45) nm, $\nu_{max.}$ (KBr) 3500, 3340, 1710, 1650, and 1592 cm^{-1}, τ ($CDCl_3$) 2·97 (1H, s, OH, exchangeable with D_2O), 2·98 (1H, q, J 6·5 and 1·5 Hz, H-6), 3·49 (1H, d, J 1·5 Hz, H-1), 3·64 (1H, d, J 6·5 Hz, H-7), 6·65 (1H, s, HO, exchangeable with D_2O), 7·01 (1H, d, J 15 Hz), 7·78 (3H, s, quinone CH_3), 8·51 (3H, s, CH_3), 8·65 (6H, s, CH_3), 8·84 (3H, s, CH_3), and 9·11 (3H, s, CH_3), *m/e* 437 (17%), 436 (55), 422 (5), 267 (4), 253 (10), 241 (41), 227 (8), 215 (14), 202 (42), 201 (100), 200 (17), 187 (6), 109 (7), 107 (7), 95 (10), 85 (23), and 83 (36).

We thank the Chemical Society for a Briggs Scholarship (to M. M.), Dr. S. D. Ward (University of Liverpool), Mr. A. McGill (Torry Research Station), and the P.C.M.U. (Aldermaston) for mass spectra, and Dr. P. M. Scopes for c.d. measurements.

[3/995 *Received*, 16*th May*, 1973]