610. *Steroids. Part XIX.* The Structure of Digifolein and Digifologenin.*

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Digifologenin is shown to be 2β,3β-dihydroxy-12α,20α-epoxy-14β,17α-pregn-5-ene-11,15-dione; it is thus 2β-hydroxydiginigenin.

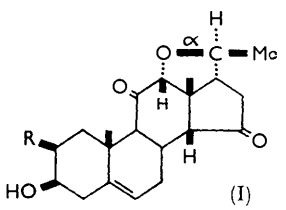
Digifolein was first described by Okada and Yamada [1] and has since been isolated by Satoh and his co-workers [2] and by Tschesche and Grimmer [3] from extracts of the leaves of *Digitalis lanata* and *D. purpurea*. Lanafolein has also been isolated by Tschesche and his co-workers [3,4] from the leaves of *D. lanata*. Tschesche and Lipp [5] have shown that digifolein is digifologenin D(+)-diginoside [3] and that lanafolein is digifologenin D(−)-oleandroside.[4,5]

We have isolated digifolein from extracts of the leaves of *D. lanata* and have re-examined it in the light of the structure of diginin and diginigenin.[6,7] Both diginin [8] and digifolein [3] have been shown to yield one mol. of D(+)-diginose on mild acid hydrolysis, together with the related aglycones diginigenin, $C_{21}H_{28}O_4$, and digifologenin, $C_{21}H_{28}O_5$. Their close similarity in physical properties and chemical behaviour has been noted by Tschesche and Buschauer [4] and discussed by Shoppee, Lack, and Robertson.[7] In

* Part XVIII, *J.*, 1962, 3624.

[1] Okada and Yamada, *J. Pharm. Soc. Japan*, 1953, **73**, 525.
[2] D. Sato, Yoshito, Ishii, and Nichimura, *Chem. and Pharm. Bull.* (*Japan*), 1953, **1**, 305, 396; D. Sato, Ishii, and Oyama, *ibid.*, 1955, **75**, 1025, 1173.
[3] Tschesche and Grimmer, *Chem. Ber.*, 1955, **88**, 1569.
[4] Tschesche and Buschauer, *Annalen*, 1957, **603**, 59.
[5] Tschesche and Lipp, *Annalen*, 1958, **615**, 210.
[6] Shoppee, Lack, and Robertson, *Proc. Chem. Soc.*, 1962, 65.
[7] Shoppee, Lack, and Robertson, *J.*, 1962, 3610.
[8] Shoppee and Reichstein, *Helv. Chim. Acta*, 1940, **23**, 975; 1942, **25**, 1611.

contrast to diginigenin, digifologenin contains two secondary hydroxyl groups which are present as a *cis*-α-glycol group readily cleaved by periodic acid and characterised by formation of an isopropylidene derivative.



(I)

Diginigenin has been shown [6,7] to be 3β-hydroxy-12α,20α-epoxy-14β,17α-pregn-5-ene-11,15-dione (I; R = H); we now show that digifologenin is 2β,3β-dihydroxy-12α,20α-epoxy-14β,17α-pregn-5-ene-11,15-dione (I; R = OH). The more strained stereoisomeric 12β,20α-epoxy-formula analogous to (I; R = OH) appears to be excluded by the relative chemical shifts for 12-H and 20-H in the nuclear magnetic resonance spectra, and by the very high wavelengths of the negative Cotton curves for digifolein and digifologenin, which require an axial OR linkage adjacent to the 11-carbonyl group.

By mass spectrometry digifologenin gave the appropriate molecular weight of 360; a peak at mass number 324 indicated the presence of two hydroxyl groups, whilst the absence of a peak at mass number 331 suggested the absence of an angular aldehyde group,[3,4] but the splitting pattern of the highly oxygenated molecule was so extensive that no further useful structural information could be derived from this spectrum.

The infrared spectra [7] of digifolein, lanafolein, and digifologenin disclose two carbonyl groups ($\nu_{max.}$ 1745, 1725 cm.$^{-1}$); one carbonyl group (reactive) must be in ring D and the other (hindered) in a six-membered ring. The maximum at 1745 cm.$^{-1}$ disappears in dihydrodigifolein ($\nu_{max.}$ 1710 cm.$^{-1}$), dihydrolanafolein (1712 cm.$^{-1}$), and dihydrodigifologenin (1716 cm.$^{-1}$), obtained [4,5] by brief treatment with sodium borohydride; both maxima disappear in tetrahydrodigifolein, and in the tetrahydrolanafoleins A, B, and C (C=C absorption, 1640 cm.$^{-1}$), obtained by prolonged reduction with sodium borohydride. The dihydro-derivatives are 11-oxo-15α-ols, whilst the tetrahydro-derivatives are 11β,15α-diols. Despite the provisional assignment [7] of the quasi-equatorial 15β-hydroxyl configuration, we now incline to the view that the quasi-axial 15α-hydroxyl orientation is more probable.

These conclusions are confirmed by the nuclear magnetic resonance spectrum of digifolein, which shows that it, like diginin,[7] does not contain an angular aldehyde group.[3,4] The spectrum discloses the presence of five methyl groups: C-18, singlet at τ 8·42 for methyl on quaternary carbon (C-13); C-19, singlet at τ 8·78 for methyl on quaternary carbon (C-10); C-21, doublet at τ 8·72 for methyl on tertiary carbon (C-20) with coupling constant J = 6·5 c./sec. typical for a freely rotating methyl group (the higher peak in the doublet overlaps the singlet signal of C-19); C-6′, doublet at τ 8·63 (J = 6·4 c./sec.) for methyl on tertiary carbon (C-5′) in the diginose residue; C-3′, singlet at τ 6·6 for methyl in the methoxyl group of the diginose residue.

The nuclear magnetic resonance spectrum of digifologenin shows the presence of three methyl groups: C-18, singlet at τ 8·42 for methyl on quaternary carbon (C-13); C-19, singlet at τ 8·78 for methyl on quaternary carbon (C-10); C-21, doublet at τ 8·72 (J = 6·5 c./sec.) for methyl on tertiary carbon (C-20), the peak of the doublet at higher field overlapping the signal of the 19-methyl group.

The corresponding nuclear magnetic resonance spectrum for diginigenin acetate [7] shows the signal for the 18-methyl group at τ 8·46 and for the 19-methyl group at τ 8·99. In the absence of data to explain the unusually low value of τ 8·46 for one of the angular methyl groups, we originally [6] allotted this signal to the 19-methyl group in diginin and diginigenin acetate, since it was allylic to the 5,6-double bond. The signal at τ 8·99 for diginin and diginigenin acetate and at τ 8·78 for digifolein and digifologenin is now assigned to the 19-methyl group. The shift of 0·21 p.p.m. in its position is due to the presence of the axial 2β-hydroxyl group. 11β-Hydroxy-steroids [9] are known to induce a paramagnetic shift of 0·20 p.p.m. in the position of the 18-methyl signal. The 2β- and the

[9] Shoolery and Rogers, *J. Amer. Chem. Soc.*, 1958, **80**, 5121; cf. Kawazoe, Y. Sato, Natsume, Hasegana, Okamoto, and Tsuda, *Chem. and Pharm. Bull.* (*Japan*), 1962, **10**, 338.

11β-hydroxyl group have the same 1,3-diaxial configuration with regard to the 19- and the 18-methyl group, respectively.

Optical rotatory dispersion (in methanol).

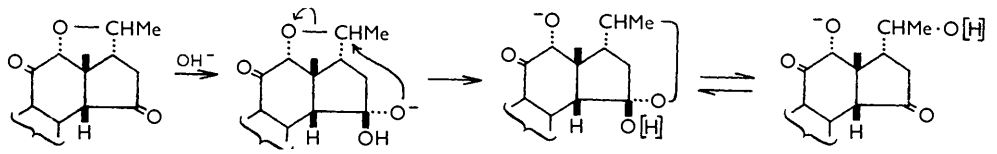
Compound	ϕ	Peak and trough λ (mμ)		Δλ	Mol. amplitude $10^{-3}a$
Digifolein	−15,500	331	}	45	−258
	+10,300	286	}		
Digifologenin	−9950	333	}	—	−>200
	Incomplete	—	}		
Dihydrodigifolein	−3180	320	}	28	−27
	−500	292	}		
Dihydrodigifologenin	−3570	327	}	37	−36
	+45	290	}		

Since the long-range shielding effects of several substituents on the protons of the 19- and the 18-methyl group are known to be additive,[9-11] it is possible, with the information now available, to calculate the approximate theoretical positions for both these groups in digifologenin. We have found that the 5,6-double bond in cholesteryl acetate and the 2β-hydroxyl group in 5α-cholestane-2β,3β-diol each lower the 19-methyl signal by 0·13 p.p.m. relative to τ 9·1 found for 5α-cholestan-3β-ol. This is substantiated by the difference of 0·13 p.p.m. for the 19-methyl signal of 5α-cholestan-2β-ol (τ 8·98) compared with that of 5α-cholestane (τ 9·1). The 11-carbonyl group also lowers the 19-methyl signal by about 0·1 p.p.m.[9] in 3β-hydroxy-5α-pregnane-11,20-dione, to place the calculated value for the 19-methyl signal in digifologenin at τ 8·74, which is in good agreement with the experimental figure of τ 8·78.

The exceptionally low τ values of 8·45 and 8·42 for the 18-methyl signals in diginigenin and digifologenin are due mainly to the presence of the 14β,17α-configuration. Dr. G. Slomp of The Upjohn Co., Kalamazoo (personal communication), has found a paramagnetic shift in the 18-methyl signal in a study of 14β,17α-steroids. The presence of the 12α,20α-oxide linkage may also have a slight effect on the position of 18-methyl signal similar to that observed on the position of the 19-methyl signal by the presence of a 1α,11α-oxide bridge.[12]

The optical rotatory dispersion characteristics of digifolein, digifologenin, dihydrodigifolein, and dihydrodigifologenin are set out in the Table. The very strong negative Cotton curves for digifolein and digifologenin are consistent with the 15-oxo-14β-structure, whilst the small negative Cotton curves, given by dihydrodigifolein and dihydrodigifologenin, show that the 15-carbonyl group which is mainly responsible for the very large Cotton effect has been reduced to a 15α-hydroxyl group.

Attention was directed [7] to the immediate reduction at 20° of ammoniacal silver solution (Tollens reagent) by diginin, diginigenin, and those of its derivatives which possess a 15-carbonyl group; similar observations have been made [3-5] for digifolein, lanafolein, and digifologenin. This capacity for reduction is clearly connected with the presence of



the 15-carbonyl group, although 15-keto-steroids do not normally act as reducing agents, and it appears to be explicable as follows. Owing to the cage structure of diginigenin and digifologenin, addition of a hydroxyl ion to the less sterically hindered β-face of the

[10] Slomp and McGarvey, *J. Amer. Chem. Soc.*, 1959, **81**, 2200.
[11] Cox, Bishop, and Richards, *J.*, 1960, 5118.
[12] Kalvoda, Anner, Arigoni, Heusler, Immer, Jeger, Mihailovic, Schaffner, and Wettstein, *Helv. Chim. Acta*, 1961, **44**, 186; Zurcher and Kalvoda, *ibid.*, p. 198.

15-carbon atom produces an anionic oxygen atom in close spatial proximity to the 17,20-bond. Successive electron transfers then generate a 11-oxo-12-oxide anion as illustrated. The 15-carbonyl group thus acts as a trigger to produce an α-ketol grouping, involving the 11-carbonyl group, which is the site of reduction. It is consistent that the colour reaction with 3,5-dinitrobenzoic acid and alkali (the Kedde reaction [13]), and the reduction of triphenyltetrazolium chloride, regarded as specific for α-ketols,[14] are positive for diginin, digifolein, lanafolein, diginigenin, and digifologenin, but negative for dihydrodigifolein, dihydrolanafolein, dihydrodiginigenin, and dihydrodigifologenin.[4,7]

The nuclear magnetic resonance spectrum of digifologenin further shows seven protons at low field which are assigned as follows: (i) 6-H, complex multiplet at τ 4·25 for one olefinic proton; (ii) 3-H, broad peak at τ 6·45 corresponding to 1 proton; (iii) 20-H, broad peak at τ 5·4 corresponding to 1 proton; (iv) 2-H, broad peak at τ 5·86 corresponding to 1 proton and placed at lower field than 3-H as it is an equatorial proton; [15] (v) 12-H, a sharp singlet at τ 6·04 for one proton on carbon attached to oxidic oxygen and flanked by carbon atoms devoid of protons; (vi and vii), a broad peak at τ 7·3 for two protons, 2,3-$(OH)_2$, which readily exchange with deuterium oxide.[16] Signal (i) confirms the presence of the double bond which is placed at position 5,6 rather than 4,5 because in the latter case the allylic 3α-proton [signal (ii)] would appear at low field, as in the case of cholest-4-en-3β-yl acetate, where the 3α-proton signal appears at τ 4·9. The signal (iii) for 20-H appears in the same position as in diginigenin acetate; [7] signal (iv) confirms the presence of the 2β-hydroxyl group, whilst the sharp singlet constituting signal (v) confirms the attachment of the oxide bridge at C-12. Signals (vi and vii) confirm the presence of two hydroxyl groups.

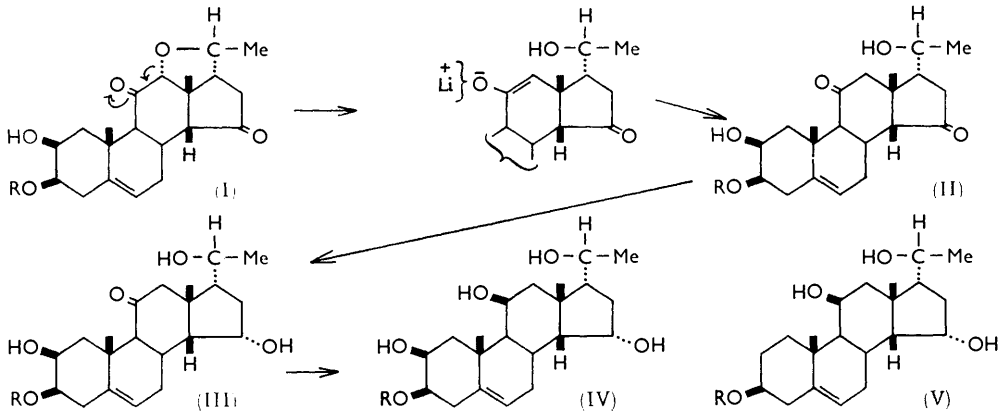
The 12α,20α-oxide bridge in digifolein (I; R = $C_7H_{13}O_3$), like that in diginigenin,[18] is unaffected by prolonged treatment with zinc and acetic acid, but is cleaved by brief treatment with lithium aluminium hydride in tetrahydrofuran, yielding three substances. One is tentatively regarded as 2β,3β,20α-trihydroxy-14β,17α-pregn-5-ene-11,15-dione 3-D(+)-diginoside (II; R = $C_7H_{13}O_3$), $\nu_{max.}$ 1735, 1710 cm.$^{-1}$ (shortage of material prevented further examination). The main product is the tetrahydroxy-ketone 3-D(+)-diginoside (III; R = $C_7H_{13}O_3$), $\nu_{max.}$ 1710 cm.$^{-1}$, whose nuclear magnetic resonance spectrum does not contain the singlet signal at τ 6·04 given by the 12β-proton in digifolein (I; R = $C_7H_{13}O_3$) and digifologenin (I; R = H). The nuclear magnetic resonance spectrum of this product reveals five methyl groups: the signal for the methoxyl group in the diginose residue appears as a singlet at τ 6·6, and the remaining four methyl groups occur as overlapping signals in a broad band between τ 8·58 and 8·77. These values indicate that there has been no shift in the position of the 19-methyl signal (τ 8·77), whilst the 18-methyl signal has been shifted (from τ 8·42) to slightly higher field by the removal of the 12α,20α-oxide bridge and reduction of the 15-carbonyl group. The third substance is the fully reduced 14β,17α-pregn-5-ene-2β,3β,11β,15α,20α-pentaol 3-D(+)-diginoside (IV; R = $C_7H_{13}O_3$), which exhibits no carbonyl absorption in its infrared spectrum.

The effect of the removal of the oxide bridge on the position of the 18-methyl group can more readily be demonstrated for diginin, where the absence of the 2β-hydroxyl group leads to less overlapping of the methyl signals. Diginin gave the 3β-D(+)-diginoside of 14β,17α-pregn-5-ene-11β,15α,20α-triol (V) as sole product when treated with lithium aluminium hydride in tetrahydrofuran.

The nuclear magnetic resonance spectrum of this product (V) reveals no change in the position of the 19-methyl signal (τ 8·91); however, although the 14β,17α-configuration

[13] Kedde, Diss., Leyden, 1946; cf. Bush and Taylor, *Biochem. J.*, 1952, **52**, 643.
[14] Kiesewalter, *Pharmazie*, 1952, **7**, 580.
[15] Zurcher, *Helv. Chim. Acta*, 1961, **44**, 1380.
[16] Jackman, " Nuclear Magnetic Spectroscopy," Pergamon, Oxford, 1959, p. 116.
[17] Ref. 16, p. 71; Fales and Robertson, *Tetrahedron Letters*, 1962, 111.
[18] Shoppee, unpublished observation.

still strongly influences the position of the 18-methyl signal, it has been shifted (from τ 8·45) to higher field (τ 8·76) by removal of the 12α,20α-oxide bridge and reduction of the 15-carbonyl group.



Experimental

For general directions see *J.*, 1959, 345; ultraviolet absorption spectra were measured for ethanolic solutions on a Perkin-Elmer 4000 A spectrophotometer; infrared absorption spectra were determined for chloroform solutions in a Perkin-Elmer model 221 double-beam instrument. Analytical samples were dried at 70°/0·5 mm. for 5 hr. Nuclear magnetic resonance spectra were determined on a Varian D.P. 60 instrument at 60 Mc./sec. with deuteriochloroform as solvent and tetramethylsilane as internal reference.

Digifolein.—Isolated from extracts of leaves of *D. lanata* by chromatography on aluminium oxide in benzene and elution with chloroform, digifolein had m. p. 198—202°, $[\alpha]_{500}$ −170°, $[\alpha]_{450}$ −235°, $[\alpha]_{400}$ −335°, $[\alpha]_{350}$ −800°, $[\alpha]_{340}$ −1350°, $[\alpha]_{331}$ −1550°, $[\alpha]_{325}$ −1335° $[\alpha]_{320}$ −900°, $[\alpha]_{313}$ −200°, $[\alpha]_{310}$ −0°, $[\alpha]_{300}$ +600°, $[\alpha]_{286}$ +1030°, $[\alpha]_{280}$ +965° [Found: C, 66·3; H, 8·05. Calc. for $C_{28}H_{40}O_8$: C, 66·6; H, 8·0%], and was homogeneous on paper chromatography with the isobutyl methyl ketone–isopropyl ether saturated with formamide and development with antimony trichloride in formamide.

Digifologenin.—Digifolein (500 mg.) in methanol (15 ml.) was treated under nitrogen with 0·5N-sulphuric acid (15 ml.) on the steam-bath for 0·5 hr. Concentration of the solution under reduced pressure followed by chloroform extraction gave an oil, which was chromatographed on aluminium oxide (15 g.) in benzene. Elution with chloroform gave amorphous digifologenin (300 mg.), m. p. 110—115°,[3-5] $[\alpha]_D$ −270° (*c* 1·0 in acetone), $\nu_{max.}$ 3610, 3560, 1733, 1710, 1140, 1092, 1070, 1052, 890, 870 cm.$^{-1}$, $\lambda_{max.}$ 310 mμ (log ε 1·9) [Found: C, 69·7; H, 8·0%; *M* (by mass spectroscopy), 360. Calc. for $C_{21}H_{28}O_5$: C, 69·95; H, 7·8%; *M*, 360]. Optical rotatory dispersion (in methanol): negative Cotton effect curve, trough $[M]_{332·5}$, −9950°; no readings could be taken below 310 mμ.

Dihydrodigifolein.—Digifolein was reduced with a solution of an old specimen of sodium borohydride (3 mol.) in aqueous 80% dioxan at 20° for 75 min., as described by Tschesche and Buschauer,[4] to give dihydrodigifolein, $\nu_{max.}$ 1710 cm.$^{-1}$; optical rotatory dispersion (in methanol): negative Cotton effect: trough, $[M]_{320}$, −3180°; peak $[M]_{292·5}$, −500°. Use of fresh sodium borohydride (3—4 mol.) in aqueous 80% dioxan at 20° for 75 min. gave tetrahydrodigifolein.

Tetrahydrodigifolein.—Digifolein (300 mg.) in methanol (6 c.c.) was treated with a solution of sodium borohydride (fresh material, 86 mg., 8 mol.) in methanol (12 c.c.) at 20° for 1 hr. Acidification with 2N-sulphuric acid to pH 3, dilution with water, and extraction with chloroform gave a product, which by crystallisation from dioxan–acetone–ether (1 : 2 : 5) at 0° furnished tetrahydrodigifolein (143 mg.), m. p. 195—198°, showing no infrared absorption maximum at 1740 or 1710 cm.$^{-1}$ (Found: C, 66·4; H, 8·4. Calc. for $C_{28}H_{44}O_8$: C, 66·1; H, 8·7%). This compound was described by Tschesche and Buschauer [4] as amorphous, $[\alpha]_D$ −10·5°, $\nu_{max.}$ 3400, 1643 cm.$^{-1}$.

Dihydrodigifologenin.—Dihydrodigifolein was hydrolysed as described by Tschesche and Buschauer [4] but under nitrogen. The product was chromatographed on aluminium oxide in chloroform; elution with chloroform gave a little amorphous material, $\lambda_{max.}$ 239 mμ (log ε 3·64), $\nu_{max.}$ 1710 (CO), 1660 (C=C·CO), 1610 cm.$^{-1}$ (C=C) regarded as dihydrodigifologenone; elution with methanol gave dihydrodigifologenin, $\nu_{max.}$ 1710 cm.$^{-1}$, previously described by Tschesche and Buschauer as having m. p. 180°, $[\alpha]_D$ −134°, $\nu_{max.}$ 3440, 1716, 1652 cm.$^{-1}$. Optical rotatory dispersion (in methanol): trough $[M]_{327·5}$, −3570°; peak $[M]_{290}$, +50°.

Reduction of Digifolein with Lithium Aluminium Hydride.—Digifolein (300 mg.) in tetrahydrofuran (20 ml.) was treated with an excess of lithium aluminium hydride (500 mg.) at 65° for 4 hr. The product extracted with chloroform was chromatographed on aluminium oxide (10 g.). Elution with chloroform gave the 2β,3β,20α-*trihydroxy*-14β,17α-*pregn*-5-*ene*-11,15-*dione* 3β-*diginoside* (25 mg.), m. p. 248—251° (from acetone–ether), $\nu_{max.}$ 3550, 1735, 1710 cm.$^{-1}$ (Found: C, 66·2; H, 8·5. $C_{28}H_{42}O_8$ requires C, 66·4; H, 8·4%). Further elution with the same solvent gave 2β,3β,15α,20α-*tetrahydroxy*-14β,17α-*pregn*-5-*en*-11-*one* 3β-*diginoside* (120 mg.), m. p. 218—221° (from acetone–ether), $\nu_{max.}$ 3608, 1710 cm.$^{-1}$ (Found: C, 65·8; H, 8·8. $C_{28}H_{44}O_8$ requires C, 66·1; H, 8·7%). Further elution with chloroform gave 14β,17α-*pregn*-5-*ene*-2β,3β,11β,15α,20α-*pentaol*-3β-*diginoside* (40 mg.), m. p. 259—260° (from acetone–ether), $\nu_{max.}$ 3610, 3600 cm.$^{-1}$ (Found: C, 66·0; H, 9·0. $C_{28}H_{46}O_8$ requires C, 65·85; H, 9·1%).

Reduction of Diginin with Lithium Aluminium Hydride.—Diginin (150 mg.) in tetrahydrofuran (10 ml.) was treated with an excess of lithium aluminium hydride (250 mg.) at 65° for 4 hr. After extraction with chloroform the product was chromatographed on aluminium oxide (5 g.). Elution with chloroform gave 14β,17α-*pregn*-5-*ene*-3β,11β,15α,20α-*tetraol* 3β-*diginoside* (110 mg.) (from acetone–ether), m. p. 258—260°, $\nu_{max.}$ 3610, 3600 cm.$^{-1}$ (Found: C, 68·3; H, 9·2. $C_{28}H_{46}O_7$ requires C, 68·0; H, 9·4%).

We thank Professor W. Klyne for the optical rotatory dispersion curves, Mr. B. Newman, B.Sc., for the preparation of dihydrodigifolein and dihydrodigifologenin, and Burroughs Wellcome & Company Limited for a gift of extracts of leaves of *D. lanata*. One of us (S. S.) acknowledges permission of Mr. H. R. Brown (Chief, Coal Research Division, C.S.I.R.O.) to participate in this work.

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