

Bark Acids of Seven *Calophyllum* Species (Guttiferae)

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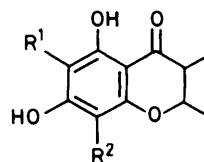
Calophyllum bracteatum, *C. calaba* (var. *calaba*), *C. moonii* (= *C. soulattri*), *C. thwaitesii*, *C. trapezifolium*, *C. walkeri*, and *C. zeylanicum* (= *C. lankaensis*) have been examined for their bark acids. A new acid, calozeyleanic acid, has been isolated from three species and has been identified as (2*R*,3*R*)-2,3-dimethyl-5-hydroxy-6-(3-methylbutyl)-6-(3,7-dimethylocta-3,6-dienyl)-8-(2-carboxy-1-phenylethyl)-2,3,6,7-tetrahydrobenzopyran-4,7-dione. Four species were found to be different, having the enantiomer of apetalic acid, isopetalic acid and a minor acid identified as the structural isomer of the latter.

Some 15 acids have been isolated¹⁻⁶ from the various parts of several *Calophyllum* species. Most of these acids possess either a phloroglucinol ring system or a cyclohexadienone (a potential phloroglucinol) system fused to a 2,3-dimethylchromanone ring (1) and (2). A majority of the acids possess the system (1).

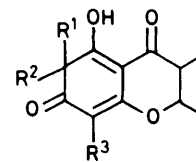
We have found in the chemical investigation of the bark acids of seven Sri Lankan endemic *Calophyllum* species that three of them namely: *C. thwaitesii* Planch and Triana, *C. walkeri* Wight; and *C. zeylanicum* Kosterm. (= *C. lankaensis* Kosterm) were similar and contained a new acid designated calozeyleanic acid (3)⁷ containing system (2) whereas the four species: *C. bracteatum* Thw., *C. calaba* var. *calaba*; *C. moonii* (= *C. soulattri*) and *C. trapezifolium* Thw. gave a different set of acids identified as the enantiomer of apetalic acid (4a), isopetalic acid (5a), and a minor acid isomeric with (5a) which has been assigned structure (6a). Both the former acids possess the ring system (1).

Several new xanthenes have been reported previously^{8,9} from these *Calophyllum* species.

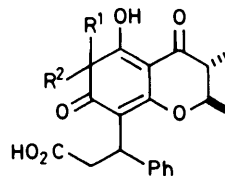
Calozeyleanic Acid (3).—The acid was isolated by preparative t.l.c. separation of the Na₂CO₃ fraction of the bark light-petroleum extractives of *C. thwaitesii*, *C. walkeri*, and *C. zeylanicum*. A molecular formula of C₃₅H₄₆O₆ (*M* 562.3309) has been assigned to calozeyleanic acid from an accurate mass measurement of the molecular ion. From the i.r. spectrum, the presence of the following groups were inferred: monosubstituted phenyl (ν_{\max} , 753 and 699 cm⁻¹), conjugated carbonyl (1 665) and hydroxy-group (3 500 and 3 200). Me₂SO₄ methylation gave a compound which had a CO₂Me (δ 3.45) and an OMe (δ 3.73) group as shown by its ¹H n.m.r. spectrum. The ¹H n.m.r. spectrum of the acid showed the presence of 5 aromatic protons (δ 7–7.4), 13 allylic protons (δ 1.4–2.0), 2 olefinic protons and a 1, one-proton low-field signal (δ 4.12) which was identified as due to a benzylic proton. Reaction of calozeyleanic acid with toluene-*p*-sulphonic acid gave a lactone (ν_{\max} , 1 775 cm⁻¹) which was identified as a new lactone, calozeyleanolactone (7), C₂₅H₂₈O₅ (*M*⁺ 408.1923), m.p. 125–126 °C, $[\alpha]_D^{25} +123.7^\circ$. The ¹H n.m.r. spectrum of this lactone showed the presence of 5 aromatic protons (δ 7.2), a triplet at δ 4.5 for a low-field benzylic proton and a doublet at δ 2.98 for 2 protons of the type CH₂CO. It also showed the presence of a chelated OH group. The lactone readily lost a C₄H₉ chain during mass fragmentation (see Scheme) and the mass spectrum showed the base peak at *m/z* 351. The high resolution of this ion showed it to have the formula C₂₁H₁₉O₅. Methylation of the lactone with Me₂SO₄ gave a monomethyl ether (δ 3.76 for OMe). The i.r. spectrum of the lactone also indicated the presence of a mono-substituted phenyl ring (760 and 699 cm⁻¹),



(1)

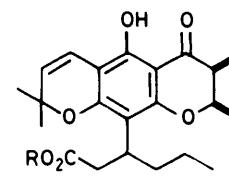


(2)



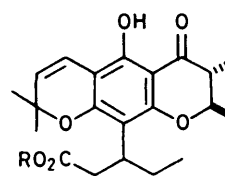
(3)

R¹ = C₅H₁₁, R² = C₁₀H₁₇



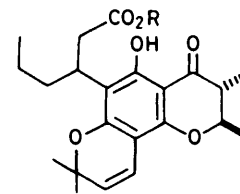
(4)

a; R = H
b; R = Me



(5)

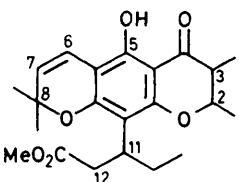
a; R = H
b; R = Me



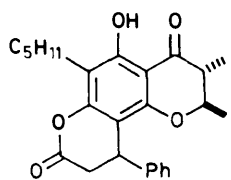
(6)

a; R = H
b; R = Me

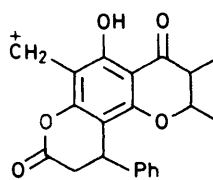
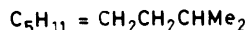
a lactone carbonyl group (1 775 cm⁻¹) and a chromanone carbonyl absorption (1 665 cm⁻¹). These data showed that calozeyleanolactone has the structure (7). The evidence for the 2,3-dimethylchromanone ring can be obtained from the chemical shifts of the 2-H and 3-H. They appeared as multiplets at δ 3.73 and 2.1 respectively. The 3-Me protons appeared as a doublet (*J* 6 Hz) centred at δ 1.10, whereas the C-2 Me protons appeared centred at δ 1.25 as a doublet (*J* 6 Hz). Due to the position of the phenyl substituent, 2-H and 3-H were found shifted to higher fields in calozeyleanolactone (and in calozeyleanic acid) than in the enantiomer of apetalic acid (4a). The 2-H,3-H coupling constant was found to be 12 Hz indicating a *trans*-diaxial orientation as in isopetalic acid

Table 1. δ Values of (4a) and (5a)


	2-H	3-H	6-H	7-H	11-H	12-H	CO ₂ CH ₃	2-Me	3-Me	8-Me	5-OH
(4a)	4.52 (m, $J_{1,2}$ 3.2 Hz)	2.4 (m)	6.56 (d, J 10 Hz)	5.38 (d, J 10 Hz)	3.64 (m)	2.61 (dd)	3.52 (s)	1.35 (d, J 6.5 Hz)	1.15 (d, J 7 Hz)	1.45 (s)	12.3 (s)
(5a)	4.13 (m, $J_{1,2}$ 12 Hz)	2.5 (m)	6.60 (d, J 10 Hz)	5.44 (d, J 10 Hz)	3.75 (m)	2.70 (dd)	3.57 (s)	1.48 (J 6.5 Hz)	1.19 (J 7 Hz)	1.43 (s)	12.5 (s)



(7)



(8)



Table 2

	a; in kg	b; in g	c; %	d
<i>C. zeylanicum</i>	3.1	56	0.42	Madugoda submontane forest
<i>C. walkeri</i>	4.15	44	0.45	Ambawela montane forest
<i>C. thwaitesii</i>	0.5	10	0.60	Kanneliya lowland forest
<i>C. trapezifolium</i>	12.0	64	0.18	Uduwela submontane forest
<i>C. moonii</i>	6.0	110	0.36	Kanneliya lowland rain forest
<i>C. calaba</i>	1.9	15	0.26	Pallai coastal lowland scrub
<i>C. bracteatum</i>	0.6	12	0.58	Kanneliya lowland rain forest

(5a). The nature of the C_5H_{11} chain in (7) remains to be elucidated. The 1H n.m.r. spectrum of (7) showed the presence of a two-proton triplet for benzylic protons at δ 2.60 and another two-proton triplet at δ 1.73. The signals for Me groups of the side chain appeared along with the chromanol ring Me groups. These and the ready elimination of the C_4H_9 chain giving rise to the base peak at m/z 351 in its mass spectrum showed that calozezylanolactone (7) has an isopentanyl side chain.

Calozezylanolactone (7), calozezylic acid (3), and tetrahydrocalozezylic acid, all gave the same base peak at m/z 351 in their mass spectra. The structure of this fragment, (8), and the molecular formula for the acid show clearly that the acid belongs to type (2). The base peak arises from the molecular ion by the loss of the chains C_4H_9 and $C_{10}H_{17}$ and a concomitant lactonisation in the mass spectrometer's probe. The fragmentation patterns are given in the Scheme. The formation of a tetrahydro-derivative when the acid (3) is hydrogenated and the presence of 13 allylic protons in the acid as shown by its 1H n.m.r. spectrum pointed to the assignment of calozezylic acid as (2*R*,3*R*)-2,3-dimethyl-5-hydroxy-6-(3-methylbutyl)-6-(3,7-dimethylocta-3,6-dienyl)-8-(2-carboxyl-1-phenylethyl)-2,3,6,7-tetrahydrobenzopyran-4,7-dione (3).

Calozezylic acid (3) isolated from the three *Calophyllum* species were found to be identical (1H n.m.r., co-t.l.c., and i.r.).

Enantiomer of Apetalic Acid (4a).—The Na_2CO_3 fraction of the bark light petroleum extractives of *C. bracteatum*, *C. calaba* var. *calaba*, *C. moonii*, and *C. trapezifolium* gave a mixture of acids. The mixture was esterified with CH_2N_2 , in each case, and the methyl esters were separated by preparative t.l.c. The more polar ester was found to be a stereoisomer of apetalic acid methyl ester (4b), $C_{23}H_{30}O_6$ (M^+ , 402.2048, requires 402.2042). The 1H n.m.r. chemical shift assignments for the protons are given in Table 1. These and other (i.r. and u.v.) data were identical with those of the methyl ester of apetalic

acid. However, the specific rotation for the methyl ester isolated was $-58^\circ C$ and hence it should be the enantiomer of the methyl ester of apetalic acid which has an $[\alpha]_D +13.4^\circ$.¹⁰

Isoapetalic Acid (5a).—The less polar methyl ester isolated from the above mixture had the formula $C_{23}H_{30}O_6$ (Found: M^+ , 402.2048, requires 402.2042) and was found to be identical with the methyl ester of isoapetalic acid (5b).¹⁰ 1H N.m.r. chemical shifts are given in Table 1.

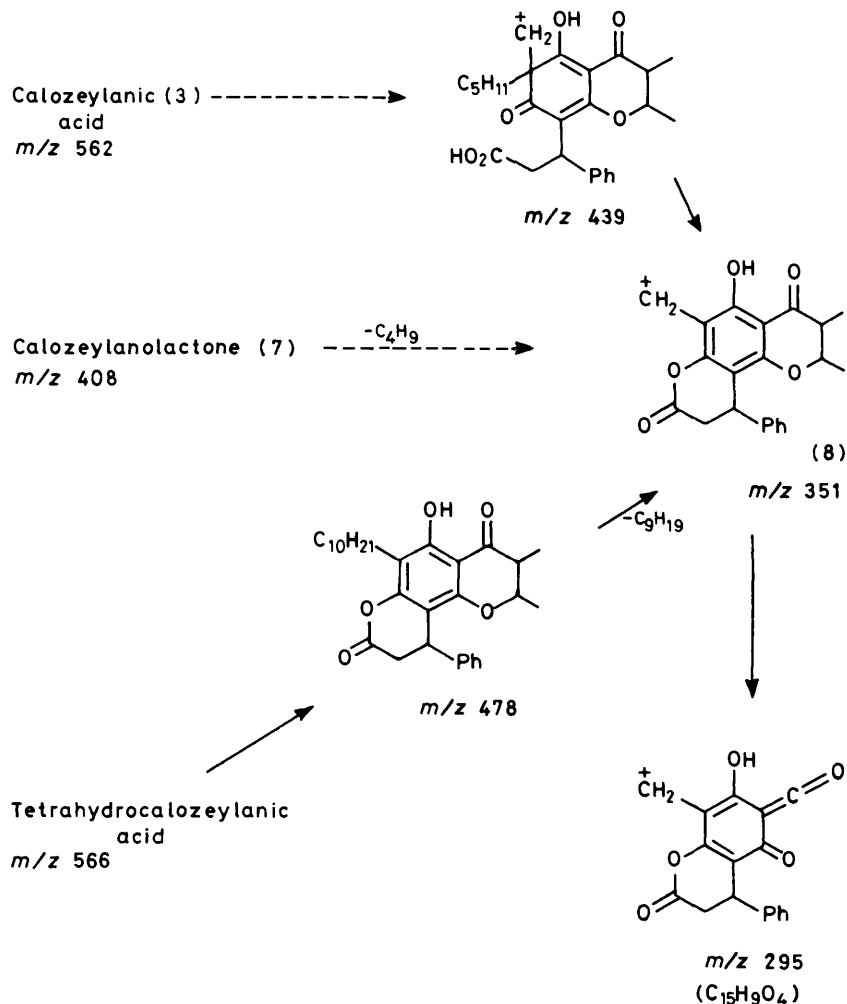
A contaminant, (6a), of isoapetalic acid (5a) which was difficult to separate from the latter even after methylation with CH_2N_2 was found in all the four *Calophyllum* species: *C. bracteatum*, *C. calaba*, *C. moonii*, and *C. trapezifolium*. The methyl ester of (6a) had a 1H n.m.r. spectrum very similar to that of (5b). The main differences being: the C-6 olefinic proton which appeared at δ 6.60 for (5b) appeared at δ 6.45; the chelated OH which appeared at δ 12.5 for (5b) appeared at δ 12.65. These data indicate that (6a) is a structural isomer of isoapetalic acid.

Experimental

The plant materials were collected in different parts of Sri Lanka. For other details see ref. 3.

Table 2 gives the weight of dry plant material (a), weight of the total light petroleum extract (b), percent weight of acid (c), and locality/vegetative type (d).

Acid refers to the Na_2CO_3 -soluble fraction of the light petroleum extracts. The Na_2CO_3 -soluble fractions were purified on a silica gel column and subsequently by preparative



Scheme. Mass fragmentations of calozeyleanic acid and its derivatives

t.l.c. (p.t.l.c.). The acids were obtained as non-crystallisable gums.

Isolation of Calozeyleanic Acid (3).—The pure acid (gum) had $[\alpha]_D +12.6$ ($CHCl_3$) (Found: M^+ , 562.3309 $C_{35}H_{46}O_6$ requires 562.3294), m/z 562 (7%), 494 (25), 476 (20), 450 (14), 439 (21), 413 (13), 407 (23), 354 (24), 353 (100), 351 (25), and 345 (12); v_{max} . ($CHCl_3$) 3 540—3 250, 1 720, 1 710, 1 665, 1 590, 753, and 699 cm^{-1} ; λ_{max} . 282 (ϵ 12 980), 362 (3 304); δ (CCl_4 , 60 MHz), 7.00—7.40 (5 H, m, aromatic), 5.15 (1 H, olefinic), 4.73 (1 H, olefinic), 4.12 (1 H, $CHPh$), 3.60 (1 H, CHO), 2.60 (2 H, m), 2.50 (2 H, m), 1.97 (2 H, m), 1.70 (9 H, $CH_3C\equiv$), and 0.8—1.4 (CH_3 and CH_2).

Calozeyleanic acid (3) was isolated from the bark extractives of *C. thwaitesii*, *C. walkeri*, and *C. zeylanicum*.

Methylation of Calozeyleanic Acid.—Calozeyleanic acid (250 mg) was dissolved in diethyl ether and was treated with CH_2N_2 . P.t.l.c. separation of the reaction mixture gave the major product as a gum (180 mg), v_{max} . 1 735, 1 660, 1 590, 1 460, 1 300, 1 170, 1 020, 850, 760, 780, and 690 cm^{-1} ; λ_{max} . 275 (ϵ 25 290) and 360 (2 810); δ (CCl_4) 7.2 (5 H, aromatic), 5.15 (1 H, olefinic), 4.77 (1 H, olefinic), 4.07 (1 H, $CHPh$) 3.81 (3 H, s, OCH_3), 3.78 (1 H, m, $CH-O$), 3.45 (3 H, s, CO_2Me), 2.8 (3 H, m), 2.5 (4 H, m), 1.3—2.0 (13 H, allylic protons), 1.2 (6 H, CH_3), 0.97 (3 H, d, J 5 Hz), and 0.78 (3 H, d, J 5 Hz).

Tetrahydrocalozeyleanic Acid.—Calozeyleanic acid (500 mg) was dissolved in EtOH (10 ml) and was hydrogenated using Pd-C catalyst. The product was purified by p.t.l.c. and the pure product was isolated as a gum, $[\alpha]_D +17^\circ$ ($CHCl_3$) (Found: M^+ , 566.3606. $C_{35}H_{50}O_6$ requires 566.3606); m/z 566 (54%), 551 (22), 548 (33), 530 (22), 520 (13), 495 (55), 478 (35), 410 (64), 409 (85), 353 (91), 351 (100), 309 (26), and 107 (38); v_{max} . (KBr) 3 400—3 600, 3 000—3 100, 1 720, 1 600—1 650, 1 330, 1 200, 920, 760, and 699 cm^{-1} ; δ ($CDCl_3$) 7—8.3 (5 H, aromatic), 4.18 (1 H, m, $CHPh$), 3.80 (1 H, m, CHO), 2.3—3.0 (low field CH_2 and CH), and 0.7—2.2 (CH_2 and CH_3).

Dimethyl Sulphate Methylation of Calozeyleanic Acid.—Calozeyleanic acid (150 mg) was dissolved in acetone (5 ml). Anhydrous K_2CO_3 (50 mg) and dimethyl sulphate (0.5 ml) were added and the mixture was heated in an oil-bath (80 °C, 6 h). The methylated product was separated by p.t.l.c. to give the pure product (102 mg) as a gum; δ ($CDCl_3$) 7.0—7.2 (5 H, aromatic), 5.15 (1 H, olefinic), 4.73 (1 H, olefinic), 4.13 (1 H, $CHPh_2$), 3.73 (3 H, s, OCH_3), 3.78 (1 H, m, CHO), 3.45 (3 H, s, CO_2CH_3), 2.3—2.5 (4 H, m), 2.1—3.0 (13 H, allylic protons), 1.2 (8 H, $2 \times CH_3$, 2 H, aliphatic), 1.0 (3 H, d, J 5 Hz), and 0.82 (3 H, d, J 5 Hz).

Calozeyleanolactone (7).—Calozeyleanic acid (200 mg) was dissolved in benzene (10 ml) and toluene-*p*-sulphonic acid

(25 mg) was added. The reaction mixture was cooled and solvent was evaporated. The residue was dissolved in ether and was shaken with water to remove excess toluene-*p*-sulphonic acid. The ether layer was dried and solvent was evaporated. The residue was purified by p.t.l.c. to give calozezylanolactone (7) (125 mg). Pure lactone was obtained by recrystallisation (light petroleum-MeOH) to give white needles, m.p. 125–126 °C, $[\alpha]_D +123.7$ (CHCl₃) (Found: M^+ , 408.1923. C₂₅H₂₈O₅ requires 408.2395), m/z 408 (48), 394 (12), 352 (43), 351 (100), 331 (12), 317 (98), 295 (70), 261 (40), 253 (54), and 149 (98); ν_{\max} (KBr) 3 240, 2 900–3 000, 1 775, 1 665, 1 440, 1 420, 1 363, 1 325, 1 230, 1 188, 1 157, 1 142, 1 110, 1 030, 970, 880, 760, and 699 cm⁻¹; λ_{\max} 238 (ϵ 5 406), 290 (4 386), 324 (1 122), and 392 nm (306); δ (CDCl₃) 14.3 (1 H, s, OH), 7.20 (5 H, m, aromatic), 4.50 (1 H, t, J 4.5 Hz), 3.73 (1 H, t, J 6 Hz), 2.98 (2 H, d, J 4.5 Hz), 2.6 (2 H, t, J 6 Hz), 2.1 (1 H, m), 1.73 (2 H, t, J 6 Hz), 1.25 (3 H, d, J 6 Hz), 1.10 (3 H, d, J 6 Hz), and 0.9–1.2 (CH₂ and CH₃).

Monomethylcalozezylanolactone.—Calozezylanolactone (70 mg) was dissolved in acetone (10 ml) and heated (12 h) after the addition of dimethyl sulphate (0.5 ml) and K₂CO₃ (100 mg).

Anhydrous conditions were maintained throughout. Acetone was evaporated and dimethyl sulphate was destroyed by adding ammonia. The product was partitioned between ether-water. Evaporation of ether gave monomethylcalozezylanolactone as a gum, δ (CDCl₃) 7.2 (5 H, s, aromatic), 4.48 (1 H, t, J 5 Hz, COCH₂CHPh₂), 3.76 (3 H, s, OMe), 2.91 (2 H, d, J 5 Hz, COCH₂CHPh₂), 2.63 (2 H, m, side chain protons), 2.1 (1 H, m), 1.22 (3 H, d, J 6 Hz, CH₃), 1.10 (3 H, d, J 6 Hz, CH₃), and 1.1 (6 H, 2 CH₃).

Isolation of the Enantiomer of Apetalic Acid (4a).—The Na₂CO₃-soluble fractions of the bark extractives of *C. bracteatum*, *C. calaba*, *C. moonii*, and *C. trapezifolium* gave a mixture of acids which were separated as the methyl esters obtained by esterifying the acid mixture with CH₂N₂. P.t.l.c. separation gave the enantiomer of apetalic acid methyl ester as a gum, $[\alpha]_D -58^\circ$ (Found: M^+ , 402.2048. C₂₃H₃₀O₆ requires M , 402.2042), m/z 402 (19%), 388 (30), 387 (100), 375 (8), 365 (8), 359 (6), 329 (10), 327 (9), 289 (9), 285 (6), and 271 (6); ν_{\max} (CHCl₃) 3 200–3 500, 1 730, 1 650, 1 580, 1 460, 1 440, 1 400, 1 380, 1 360, 1 340, 1 280, 1 200, 1 160, 1 120, 1 020, 920, 890, 750, and 660 cm⁻¹; λ_{\max} (EtOH) 228 (log ϵ 3.59), 267 (4.62), 312 (4.02), and 363 nm (3.32); δ (CCl₄) 12.3 (chelated OH), 6.56 (1 H, d, J 10 Hz), 5.38 (1 H, d, J 10 Hz), 4.52 (1 H, m, J 3.2 Hz), 3.64 (1 H, m), 3.52 (3 H, s,

CO₂CH₃), 2.61 (2 H, dd), 2.4 (1 H, m), 1.45 (6 H, s, 2CH₃), 1.35 (3 H, d, J 6.54 Hz), and 1.15 (3 H, d, J 7 Hz).

Isolation of Isoapetalic Acid (5a).—P.t.l.c. separation of the CH₂N₂ reaction product of the above acid mixture gave methyl ester of isoapetalic acid as a gum, $[\alpha]_D -91^\circ$ (lit.¹⁰ -68.3°) (Found: M^+ , 402.2048. C₂₃H₃₀O₆ requires 402.2042), m/z 402 (21%), 388 (25), 287 (100), 359 (3), 329 (1), 313 (7), 285 (15), 271 (12), 257 (11), 243 (9), and 229 (15); ν_{\max} (CHCl₃) 2 900, 1 740, 1 660–1 640, 1 593, 1 475, 1 465, 1 415, 1 400, 1 369, 1 307, 1 280, 1 176, 1 160, 1 140, 1 097, 1 070, 1 025, and 907 cm⁻¹; λ_{\max} (EtOH) 228 (log 4.01), 268 (4.42), 274 (4.63), 301 (4.02), 315 (4.01), and 371 nm (3.62); δ (CCl₄) 12.50 (1 H, s, chelated OH), 6.60 (1 H, d, J 10 Hz), 5.44 (1 H, d, J 10 Hz), 4.13 (1 H, m, J 12 Hz), 3.75 (1 H, m), 3.57 (3 H, s, CO₂CH₃), 2.70 (2 H, dd), 2.5 (1 H, m), 1.48 (3 H, d, J 6.5 Hz), 1.43 (6 H, s, 2CH₃), 1.19 (3 H, d, J 7 Hz), and 0.88 (3 H).

The identity of the methyl ester of isoapetalic acid was confirmed by comparison with an authentic sample and by preparation of its acetate.

Acknowledgements

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