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CONSTITUENTS OF THAI MEDICINAL PLANTS—I AGLAIOL

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Abstract—Five crystalline compounds have been isolated from the leaves of the oriental medicinal plant *Aglaia odorata* Lour. (Meliaceae). One of these (aglaiol) is a tetracyclic triterpene, $C_{30}H_{50}O_2$, which according to chemical reactions and spectra is shown to possess structure I. This formula was confirmed by correlation with a derivative from dammaradienyl acetate (VII).

Aglaia odorata Lour. (generally listed as a genus of the family Meliaceae[1]) is a small tree found predominantly in Thailand, Malaya, China and the Philippines. It is commonly known as Pra-yong or Hom-glai. Formerly, the Thai people used a water extract from the roots and leaves as a heart stimulant and febrifuge. From the dried powdered leaves of this plant, five crystalline compounds have now been isolated (Experimental), and the evidence establishing structure I for one of these compounds, which has been named aglaiol, is reported in the present paper.

The analysis of aglaiol, m.p. 113–114°, $[\alpha]_D$ +53°, fitted best the molecular formula $C_{30}H_{50}O_2$, which was eventually confirmed by the chemistry of the compound described below. In the UV spectral region aglaiol exhibited only the rising end absorption of a single di-substituted double bond, ϵ_{210} 1175.[2] The IR spectrum confirmed the presence of a double bond (ν_{max} 3050 and 1640 cm^{-1}) probably present as a methylene group (ν_{max} 887 cm^{-1}), and revealed hydroxyl absorption (ν_{max} 3600 and 3450 cm^{-1}).[3] The NMR spectrum (Fig. 1) contained a broad peak at 4·76 ppm (2 H) due to two vinyl hydrogen atoms; an even broader multiplet at ~3·23 ppm (1 H) was assigned to a proton on a carbon atom bearing oxygen. Further upfield was a symmetrical triplet (J = 6 c/s) at 2·75 ppm (1 H) caused by a second proton on carbon bearing oxygen. The methyl region had five peaks at 0·77, 0·85–0·87, 0·98, 1·27 and 1·30 ppm which were shown by integration to arise from seven methyl groups. Integration of the entire spectrum gave ~46 hydrogen atoms.

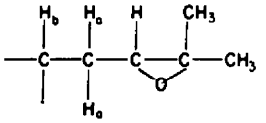
Consideration of this information strongly implicated a triterpene (30 carbon atoms including 7 methyl groups) with a side chain to permit the presence of the methylene group. The usual 3β-hydroxyl group was probably present since the 3·23 ppm peak (1 H) was in the same position as the 3α-hydrogen of other 3β-hydroxytriterpenoids examined in these Laboratories. The unusually low field position (1·27 and 1·30 ppm) of two of the methyl groups could be accounted for by the presence of the

[1] [a] J. D. Hooker, *Flora of British India*, Vol. I, p. 554. L. Reeve and Co., London (1875);
[b] A. Engler and K. Prantl, *Die Naturlichen Pflanzenfamilien* (First Edition) III Teil, Abteilung 4, p. 299. W. Engelmann, Leipzig (1897).

[2] P. S. Ellington and G. D. Meakins, *J. Chem. Soc.* 697 (1960).

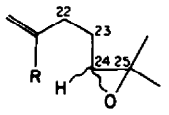
[3] L. J. Bellamy, *The Infrared Spectra of Complex Molecules* (Second Edition) Methuen, London (1958).

second oxygen atom on the carbon to which they were bound. This second oxygen atom must then be a cyclic ether to account also for the 2·75 ppm triplet (1 H). The symmetry of this triplet suggested that the single hydrogen on the carbon bearing oxygen was coupled *only* to one adjacent methylene group which was free to rotate about the connecting C—C bond, and furthermore, that any protons attached to the next carbon atom were appreciably chemically shifted from the methylene protons as in A. These conditions are readily satisfied by a modified isoöctenyl side chain, B, in which the allylic C-22 hydrogens are chemically shifted from the C-23 methylene protons. To account for the apparent absence of any other double bonds in the molecule, aglaiol should thus be a dammarane derivative, more specifically I, provided, of course, that it did belong to a known group of triterpenes.[4]



$\delta H_a \not\approx \delta H_b$

A



B

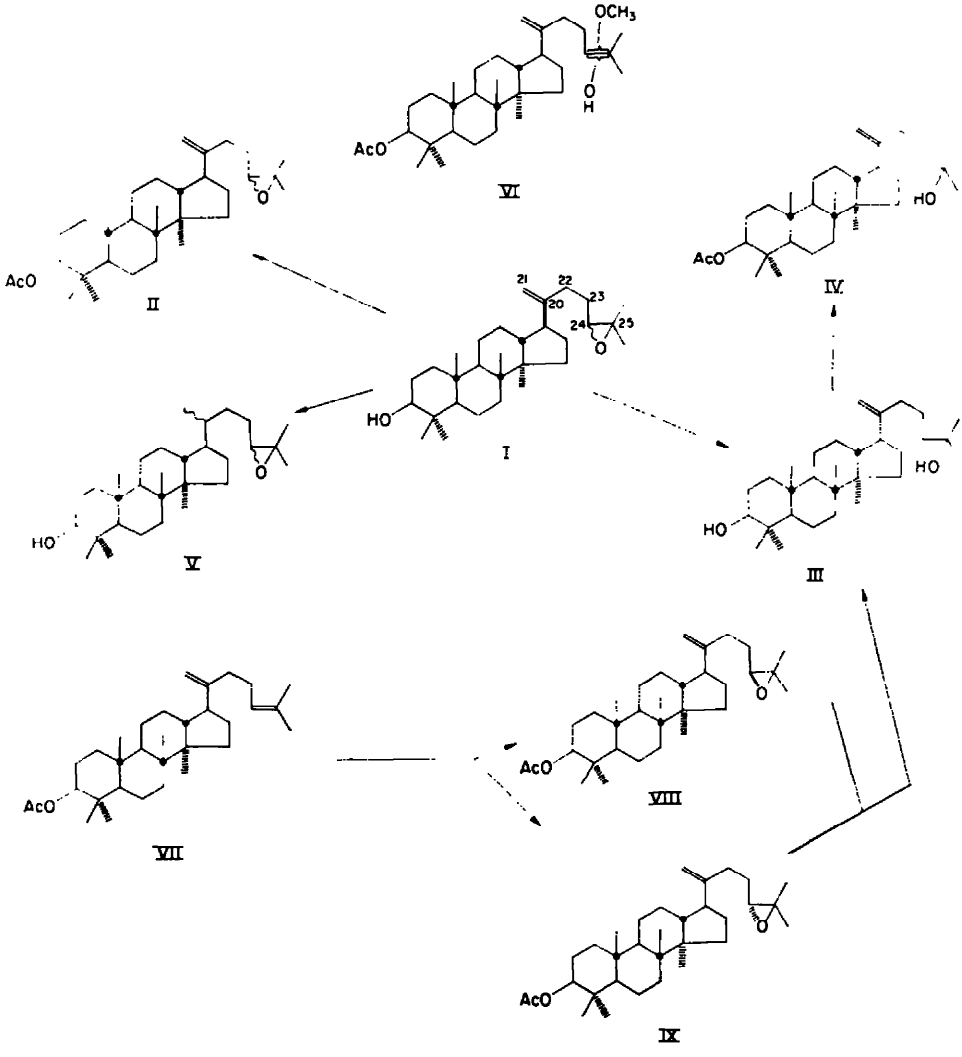
Support for this formulation was provided by the three high field methyl NMR resonance positions which correspond with those reported for the five fused ring quaternary methyl groups of analogous dammarane derivatives.[5] Chemical evidence for the structure I was obtained from the following reactions. Acetylation of aglaiol at room temperature gave a monoacetate (II) as the only product. Its IR spectrum contained an acetate carbonyl peak but no hydroxyl absorption. In the NMR spectrum the acetate methyl group appeared at 2·02 ppm and the 3α-hydrogen had undergone the characteristic shift to ~4·50 ppm.[6] When the acetylation was conducted in boiling acetic anhydride, in addition to the monoacetate, a small amount of a methoxy acetate was isolated, probably VI from acid-catalyzed scission of the oxide ring during recrystallization from methanol.

Hydrogenation of aglaiol in ethanol over palladium gave a dihydrocompound (V) which no longer contained a double bond; its IR spectrum lacked the peaks at 3050, 1640 and 887 cm^{-1}. The two vinyl hydrogen atoms had disappeared from the NMR spectrum and an eighth methyl group was found to be present by integration. The peak at ~2·68 ppm (1 H) was still present but it was no longer a symmetrical triplet since now $\delta_{H_a} \approx \delta_{H_b}$ in A.

Aglaiol was reduced by LAH to a dihydrocompound whose IR spectrum had increased hydroxyl absorption as well as the peaks at 3060, 1640 and 887 cm^{-1} due to the doubly bonded methylene group. The NMR spectrum of LAH-dihydroaglaiol still contained the broad peak (2 H) from the vinyl hydrogens at 4·72 ppm and the broad multiplet at ~3·20 ppm due to the 3α-proton, but the triplet centered at 2·75 ppm (I H) was missing as would be the case if an epoxide had been reductively cleaved at C-24 to yield III. Acetylation of LAH-dihydroagaliol at room temperature with acetic anhydride and pyridine produced a monoacetate whose IR spectrum had

[4] G. Ourisson and P. Crabbé, *Les Triterpènes Tétracycliques* Hermann, Paris (1961).
[5] J.-M. Lehn, *Bull. Soc. Chim. Fr.* 1832 (1962).
[6] L. M. Jackman, *Nuclear Magnetic Resonance Spectroscopy* p. 55. Pergamon Press, Oxford (1959).

hydroxyl as well as acetate carbonyl absorption. Since the conditions used are those for acetylation of primary and secondary alcohols, the monoacetate was assigned the structure IV which was confirmed by shift of the 3α-proton from 3·20 ppm in III to ~4·48 ppm in the NMR spectrum of the monoacetate.



With chemical and physical evidence for the functional groups and presumptive evidence for the skeleton of I, it was decided to substantiate this structure by a partial synthesis from a known dammarane derivative. To this end dammaradienyl acetate (VII) was epoxidized with one equivalent of monoperphthalic acid. Although the major product was the epoxide of the trisubstituted double bond (presumably a mixture of VIII and IX, each having the same R_f on TLC), and although it crystallized readily in beautiful needles, a pure compound could not be obtained even on seeding with aglaiol monoacetate (II). Therefore, the entire peracid product was reduced with LAH to eliminate isomerism at C-24. From the reduction product was separated 35% of a component which after recrystallization was identical in all respects (m.p.,

mixture m.p., $[\alpha]_D$, IR, NMR, TLC) with LAH-dihydroaglaiol (III). This correlation with a known dammarane triterpene provides proof of the structure and stereochemistry of aglaiol (I) except for the configuration at C-24. Although isolated from an entirely different plant, aglaiol is a close relative of protopanaxadiol[7] and ocotillol[8] as well as the other dammarane compounds possessing the usual hydroxyisoöctenyl side chain.[4]

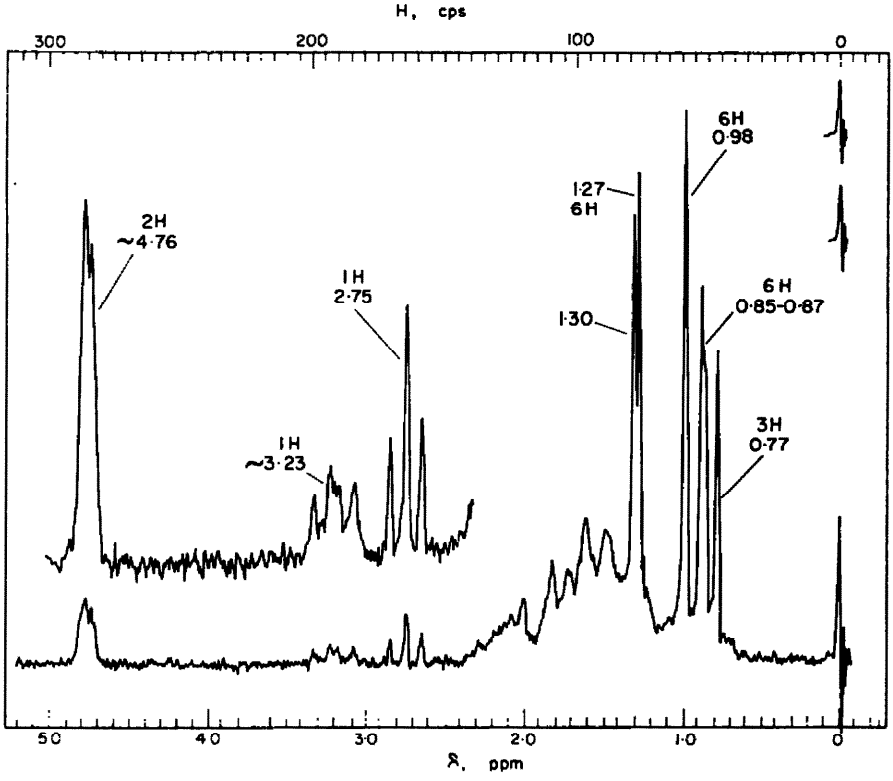


FIG. 1. NMR Spectrum of Aglaiol.

EXPERIMENTAL

General. M.ps are uncorrected and were taken on a Fisher–Johns hot stage; those marked micro m.p. were taken on a microscope hot stage. Optical rotations were observed on $CHCl_3$ solutions in a 1- or 2-dm tube with a Schmidt & Haensch polarimeter (15364). UV spectra were taken on a Bausch and Lomb Spectronic 505 instrument. IR spectra were recorded with a Beckman IR-5 spectrometer. NMR spectra were determined in $CDCl_3$ solution on a Varian A-60 spectrometer. Integrations were done by the cut-and-weight method. Silica gel with $CaSO_4$ binder and $CHCl_3$ containing 0·75% EtOH were used for thin layer chromatography. R_f values reported were taken from a single plate on which all compounds were present. Pet. ether refers to the fraction b.p. 40–80°. Microanalyses were carried out by Dr. K. W. Zimmermann and associates, C.S.I.R.O., Carlton, Victoria, Australia, and by Dr. C. Daesslé, Montreal, Quebec, Canada.

Extraction of Aglaia odorata *and separation of components*

Ground dried *Aglaia odorata* leaves (2 kg) gathered from orchards in Bangkok and Dhonburi were steeped in pet. ether (11 l.) for 7 days at room temp and then filtered. The extraction was

[7] S. Shibata, O. Tanaka, M. Sado and S. Tsushima, *Tetrahedron Letters* No. 12, 795 (1963).
[8] C. M. M. Halls and E. W. Warnhoff, *Chemistry and Industry*, 1986 (1963).

TABLE 1. NUCLEAR MAGNETIC RESONANCE ABSORPTION OF AGLAIOL DERIVATIVES*

Compound	4β-Me	4α-Me	8β-Me	10β-Me	14α-Me	25-diMe	3α-H	24-H	21=CH_2	3-O$\overset{O}{\overset{\|}{C}}CH_3$
Agliaol I	0·87 or 0·85	0·77	0·98	0·98	0·85 or 0·87	1·27 and 1·30	~3·23 m	2·75 t J=6	4·76 bs	—
Aglaiol monoacetate II	0·85	0·85	0·98	0·85	0·85	1·27 and 1·30	~4·50 m	2·74 t J=6	4·75 bs	2·02
H_2-Dihydroaglaiol V	0·85	0·76	0·97	0·97	0·85	1·28	~3·18 m	~2·68 m	—	—
LAH-Dihydroaglaiol III	0·87	0·78	0·97	0·97	0·87	1·21	~3·20 m	—	4·72 bs	—
LAH-Dihydroaglaiol monoacetate IV	0·85 or 0·86	0·85 or 0·86	0·98	0·85 or 0·86	0·85 or 0·86	1·21	~4·48 m	—	4·72 bs	2·02
Methoxyaglaiol monoacetate VI	0·86	0·86	0·98	0·86	0·86	1·11	~4·51 m	~2·4 m 3·22 OMe	4·74 bs	2·02
Dammaradienyl acetate VII	0·86	0·86	0·97	0·86	0·86	1·61 and 1·67	~4·48 m	~5·12 m	4·72 bs	2·02

* Chemical shifts are given in ppm from tetramethylsilane (=O) for deuterochloroform solutions (conc. range ~ 10–40 mg/0·10 ml) t = triplet, b.s. = broad "singlet", m = complex multiplet.

repeated twice with fresh pet. ether. Evaporation of the filtrates almost to dryness on the steam bath left 77 g (3·8%) of a greenish oily residue.

The extracted ground leaves were next steeped in ether (10 l.) for 7 days before filtration. This extraction was also repeated with another fresh portion of ether. Evaporation of the ether filtrates gave 42 g (2·1%) of oily material.

A 17-g portion of the pet. ether extract was chromatographed on a 2-cm by 45-cm column of Merck alumina. The following Table summarizes the results obtained after recrystallization of fractions.

Volume	Eluent	Wt.	Eluate
1·5 l.	pet. ether	823 mg	wax
1 l.	ether:pet. ether (1:9)	1·235 g	yellow oil
1·5 l.	ether:pet. ether (1:4)	283 mg	white crystals, m.p. 89–90°
		334 mg	white crystals, m.p. 113–114°
1 l.	ether:pet. ether (1:1)	12 mg	colourless needles, m.p. 133–134°
1 l.	ether:pet. ether (3:1)	37 mg	white crystals, m.p. 176–178°

After several recrystallizations from pet. ether, the first crystalline compound eluted had m.p. 89–90°. (Found: C, 82·54 82·24; H, 13·92, 13·86; O, 3·54, 3·90. $C_{30}H_{50}O$ (436·78) requires: C, 82·49; H, 13·85; O, 3·66%.)

Alumina chromatography of the ether extract gave a crystalline compound, m.p. 217–218°, whose chemistry is being investigated.

Aglaiol (I)

The mother liquors from recrystallization of the 89–90° compound on evaporation at room temp deposited a second crop of crystals. Filtration and several recrystallizations from pet. ether afforded 334 mg (1·9%) of small white felted needles of *aglaiol*, m.p. 113–114°, $[\alpha]_D^{30}$ +53° (*c* 0·174), $[\alpha]_D^{27}$ +50° (*c* 2·05). The compound gave a single spot, R_f 0·41, on thin layer chromatography in MeOH:$CHCl_3$ (1·5:98·5). (Found: C, 81·24; H, 11·36; O, 7·14; Mol. wt., 503 (Rast). $C_{30}H_{50}O_2$ (442·70) requires: C, 81·39; H, 11·38; O, 7·23%.) UV spectrum: ϵ_{210}^{EtOH} 1175, rising end absorption only; IR spectrum: $\nu_{max}^{CS_2}$ 3600 and 3450 (OH), 3050 (vinyl H), 1640 (C=C), and 887 cm^{-1} (C=CH_2).

Aglaiol decolourized Br_2 in CCl_4. The Liebermann test[9] with Ac_2O and H_2SO_4 gave a red colour that changed to brown on standing. If $CHCl_3$ was used as solvent a purple colour appeared which changed to greenish-brown. In the Tschugaeff colour test[10] with HOAc, AcCl and $ZnCl_2$ a pink colour was obtained which gradually changed to red and then to reddish brown. In the Rosenheim colour test[11] a pink colour appeared overnight.

Acetylation of aglaiol (I)

(a) A mixture of aglaiol (300 mg), acetic anhydride (2 ml) and pyridine (5 ml) was allowed to react at room temp for 24 hr. On being poured into water the acetylation product crystallized and was removed by filtration. Recrystallization from MeOH gave 272 mg (82%) of small colourless needles of *aglaiol monoacetate* (II), m.p. 161–162°, $[\alpha]_D^{30}$ + 67° (c 0·199). The compound gave a single spot, R_f 0·56, on thin layer chromatography in MeOH:$CHCl_3$ (1·5:98·5). (Found: C, 79·59; H, 10·75; O, 7·66. $C_{32}H_{52}O_3$ (484·74) requires: C, 79·28; H, 10·81; O, 9·90%). IR spectrum: $\nu_{max}^{CS_2}$ 3060 (vinyl H), 1735 (acetate C=O), 1640 (C=C), 1240 (C—O—C), and 887 cm^{-1} (C=CH_2).

(b) A solution of aglaiol (300 mg) in acetic anhydride (7 ml) was refluxed for 0·5 hr. After cooling to room temp, the reaction mixture was poured into water, and the solid product filtered off and washed with water. Recrystallization from MeOH gave 254 mg (77%) of the monoacetate II, m.p. 161–162°.

[9] C. Liebermann, *Ber. Dtsch. Chem. Ges.* **18**, 1803 (1885).
[10] L. Tschugaeff, *Chem. Ztg.* **24**, 542 (1900).
[11] O. Rosenheim, *Biochem. J.* **23**, 47 (1929).

From the mother liquors were deposited 32 mg of small white prisms of a methoxy acetate, probably VI, m.p. 140–141°. The compound gave a single spot, R_f 0·49, on thin layer chromatography in MeOH:$CHCl_3$ (1·5:98·5). (Found: OCH_3, 5·17. $C_{33}H_{56}O_3$ (516·78) requires OCH_3, 6·00%). IR spectrum: $\nu_{max}^{CS_2}$ 3570 (OH), 3060 (vinyl H), 1735 (acetate C=O), 1635 (C=C), 1240 (C—O—C) and 885 cm^{-1} (C=CH_2).

LAH-Dihydroaglaiol (III)

A solution of aglaiol (282 mg) in anhydrous ether (10 ml) was added dropwise with stirring (magnetic bar) to a solution of LAH (5 g) in ether (100 ml) while the temp was maintained below 10°. The reaction mixture was stirred for 2 hr after addition was complete and then decomposed with dil. H_2SO_4. The ether layer was separated, and the aqueous layer extracted with several fresh portions of ether. The combined ethereal extracts were washed with $NaHCO_3$ solution, water and dried (Na_2SO_4). Evaporation of the filtered solution and recrystallization of the residue from MeOH gave 231 mg (82%) of small colourless scales of III, m.p. 143–144°, micro m.p. 145–147°, $[\alpha]_D^{30}$ +51° (*c* 0·136), $[\alpha]_D^{27}$ + 55° (*c* 1·30). The compound gave a single spot, R_f 0·19, on thin layer chromatography in MeOH:$CHCl_3$ (1·5:98·5). (Found: C, 81·67, 81·32; H, 12·02, 11·42; O, 6·31; active H, 0·52. $C_{30}H_{52}O_2$ (444·72) requires: C, 81·02; H, 11·79; O, 7·20; active H, 0·45%). IR spectrum: $\nu_{max}^{CS_2}$ 3600 and 3360 (OH), 3060 (vinyl H), 1640 (C=C), and 887 cm^{-1} (C=CH_2).

H_2-*Dihydroaglaiol* (V)

A solution of aglaiol (200 mg) in absolute EtOH (5 ml) was hydrogenated at room temp and atm. press. in the presence of Pd-C (10 mg) until uptake of H_2 ceased. The catalyst was removed by filtration, the solution evaporated to dryness, and the residue chromatographed on a 1-cm × 15-cm column of Merck alumina. Ether: pet. ether (1:4) eluted V which was recrystallized from pet. ether to give 126 mg (63%) of small colourless prisms, m.p. 115–116°, $[\alpha]_D^{30}$ +57° (*c* 0·124). The compound gave a single spot, R_f 0·44, on thin layer chromatography in MeOH:$CHCl_3$ (1·5:98·5). (Found: C, 81·02; H, 11·59; O, 7·39. $C_{30}H_{50}O_2$ (444·72) requires: C, 81·02; H, 11·79; O, 7·20%.) IR spectrum: $\nu_{max}^{CS_2}$ 3600 and 3460 cm^{-1} (OH).

LAH-Dihydroaglaiol monoacetate (IV)

A mixture of LAH-dihydroaglaiol (III) (200 mg), acetic anhydride (2 ml) and pyridine (5 ml) was allowed to react at room temp for 24 hr. On being poured into water the acetylation product crystallized and was collected on a filter. Recrystallization from MeOH gave 151 mg (69%) of small colourless prisms of IV, m.p. 138–139°, $[\alpha]_D^{30}$ +69° (*c* 0·116). The monoacetate gave a single spot, R_f 0·42, on thin layer chromatography in MeOH:$CHCl_3$ (1·5:98·5). (Found: C, 78·88; H, 10·45; O, 10·67. $C_{32}H_{54}O_3$ (486·75) requires: C, 78·96; H, 11·18; O, 9·86%). IR spectrum: $\nu_{max}^{CS_2}$ 3600 and 3500 (OH), 3070 (vinyl H), 1735 (acetate C=O), 1640 (C=C), 1240 (C—O—C), and 886 cm^{-1} (C=CH_2).

Monoepoxidation of dammaradienyl acetate (VII)

To a solution of pure VII (275 mg; 0·58 mmole) in reagent grade ether (6 ml) was added an ethereal solution of monoperphthalic acid (1·40 ml; 0·57 mmole of peracid). The reaction mixture was allowed to stand at 10° for 22 hr and at room temp for 20 hr. The solution was then poured into water and extracted with 3 portions of ether. The combined ethereal solutions were washed with water, $NaHCO_3$ solution, water, sat. NaCl solution and dried ($MgSO_4$). Filtration and evaporation of ether left 292 mg (103%) of white solid. Recrystallization from ethyl acetate or from 95% EtOH gave long colourless needles, micro m.p. 164–171°, unchanged on further recrystallization. Thin layer chromatography with MeOH:$CHCl_3$ (1·5:98·5) revealed the crystals to be a mixture of at least 3 or more likely 4 compounds (VII, VIII, IX, and probably some bis-epoxide).

Synthetic LAH-dihydroaglaiol (III)

The crystals and mother liquor residues from the preceding experiment were combined (290 mg) in dry tetrahydrofuran (25 ml). LAH (0·50 g) was added and the reaction mixture refluxed for 17 hr. The reaction mixture was worked up essentially as described for the preparation of LAH-dihydro-aglaiol. Evaporation of the organic solvent left 307 mg of oily residue. Although the major component

of the product was shown by thin layer chromatography to be III, this diol could not be induced to crystallize even on seeding with LAH-dihydroaglaiol. Therefore the mixture was separated by thick layer chromatography on three 20 × 20-cm plates containing 20 g of silica gel per plate. Arbitrary separation of the silica gel layers into zones and extraction with $CHCl_3$–MeOH gave several fractions. Those consisting of almost pure III, according to thin layer chromatography, were combined (92 mg, 35% from VII) and recrystallized twice from ethyl acetate–pet. ether to give 17 mg of colourless prisms, micro m.p. 145–147° with sintering at 144°, $[\alpha]_D^{27}$ +51° (*c*, 1·56). The compound gave a single spot, R_f 0·19, on thin layer chromatography in MeOH:$CHCl_3$ (1·5:98·5). (Found C, 80·84; H, 11·32. $C_{30}H_{52}O_3$ (444·72) requires: C, 81·02; H, 11·79%).

The compound was identical in all respects (m.p., $[\alpha]_D$, TLC, IR, NMR) with the hydride reduction product III of aglaiol. A mixture of the two melted at 143–147°, undepressed.

Acknowledgements—We wish to express our thanks to Professor Kasin Suvatabandhu for help in obtaining the *Aglaia odorata* leaves and to Dr. K. Jewers for helpful discussions. We would also like to thank Dr. J. S. Mills for a generous gift of dammaradienyl acetate.