amount of pelargonidin 3-glucoside was formed in addition to pelargonidin 3-galactoside on hydrolysis of II, suggested that very little of the latter was hydrolysed during extraction and purification.

Hydrolysis of I and II with 10% HOAc or H_2O_2[5] (including a mild treatment for only 2 hr without the final ammonia addition) gave galactose and glucose. Since I and II appeared to contain biosides which were unstable when subjected to these treatments, tests were made with α- and β-linked biosides containing glucose and galactose. Lactose (β) solabiose (β), lycobiose (β) and melibiose (α) were unaffected by H_2O_2 omitting the final treatment with NH_4OH, but some breakdown to glucose and galactose was observed when this treatment was given.

The minor components observed during final solvent development in BAW were artifacts[7] produced by the combined action of HCl (carried over on the paper from the preceding development in HOAc–HCl) and acetic acid (from the eluting solvent MAW). They contained identical aglycone and sugar components to their corresponding major anthocyanins and were reconverted to them by cold HCl. Thus, although the artifact of I formed a single spot (R_f 0·40) in BAW without HCl in the applied solution, a small amount of I (R_f 0·25) separated when HCl (1%) was present in the applied solution. With increase in acid concentration (up to 10%) the amount of I increased; at the same time the R_f value of the artifact became less and approached I.

Six bushes were sampled including an authenticated bush from Bristol University Botany Department. Those with large red-pink petals contained more of the cyanidin than the pelargonidin glycosides; the smaller orange-pink petals contained relatively more of the pelargonidin derivates. Authentic samples of pelargonidin and cyanidin 3-sophorosides for comparison purposes were obtained from *Papaver rhoeas* petals.

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MONOCOTYLEDONAE

ORCHIDACEAE

NONSAPONIFIABLE CONSTITUENTS OF ORCHIDS: *ARUNDINA* AND *CATTLEYA*

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Abstract—The nonsaponifiable fraction of flowers and stems of the terrestrial orchid *Arundina* and leaves and stems of mixed *Cattleya* species were examined for nonsaponifiable constituents. Common to both, in the organs examined, were campesterol, stigmasterol, and β-sitosterol. In *Arundina* these sterols occurred largely as the mixed glycosides. A more detailed study of *Cattleya*, mainly the leaves, indicated a series of saturated long chain alcohols as the major constituents, and cycloeucalenol, cycloartenol, and 24-methylene cycloartanol as minor triterpenoid constituents.

INTRODUCTION

THE NONSAPONIFIABLE constituents of Orchidaceae have not, to the best of our knowledge, been subjected to chemical investigation. Consequently we undertook an investigation of such constituents of two genera of this large family: *Arundina* and *Cattleya*. According to

Holtum, "The whole *Arundina* population of Malaya is a complex mixture".[1] On this account, no attempt was made to identify the particular species under study. *Cattleya* is also extraordinarily large and complex. Therefore for examination a mixture of various hybrids was selected at random.

RESULTS AND DISCUSSION

Chromatography of Nonsaponifiable Extracts

Arundina yielded β-sitosterol as the only identifiable free sterol. It was identified utilizing TLC, GLC, and mass spectral (MS) analysis. The mass spectrum obtained was identical with a spectrum obtained from authentic β-sitosterol. It should be noted that this does not preclude the possible presence of clionasterol. However, it is doubtful that clionasterol would be present since it has been found only in algae[2] and has not yet been reported in higher plants. Acid hydrolysis of the glycoside fraction (m.p. 293–295°) eluted from the alumina column with ethanol yielded a mixture containing β-sitosterol, stigmasterol, and campesterol, in the ratio 90.7·3, as identified by TLC and GLC. The sugar moiety was not identified.

Cattleya yielded a considerable amount (64·7% of the total nonsaponifiable fraction) of a waxy solid mixed with the sterol fraction. The m.p. of this waxy material (45–85°) could not be improved by repeated crystallization. The reason for this became apparent when the sample was subjected to GLC and GLC-MS analyses. Both techniques indicated the presence of a series of saturated long chain alcohols; a C_{24} alcohol (M^+-18 = 336) accounting for 25% of the mixture; a C_{26} alcohol (M^+-18 = 364) accounting for 26%; a C_{28} alcohol (M^+-18 = 392) accounting for a further 26% of the mixture; and a C_{30} alcohol (M^+-18 = 420) which accounted for 11% of the mixture. The first component of this mixture had a retention time (t_R) equal to that of lignoceryl alcohol (tetracosanol). All the components of this mixture exhibited a linear relationship when the log of their retention time was plotted against C number. This evidence coupled with mass spectral evidence indicates the nature of these compounds as saturated long chain alcohols. Saturated long chain alcohols are commonly found in plant leaf waxes.[3]

Having crystallized out the major portion of long chain alcohols, the sterols in the nonsaponifiable extract of *Cattleya* were elucidated utilizing TLC, preparative TLC, GLC, and GLC-MS. GLC and GLC-MS of the 4αmethyl region confirmed the presence of cycloeucalenol. The mass spectrum exhibited a parent ion at *m/e* 426 with other peaks at 411(M-CH_3), 408(M-HOH), 393(M-CH_3+HOH), 365, 353, 343, and 300. GLC and GLC-MS of the 4,4-dimethyl region confirmed the presence of cycloartenol and 24-methylene cycloartanol. The peak corresponding to cycloartenol exhibited a parent ion at *m/e* 426 with other peaks at 411(M-CH_3), 408(M-HOH), 393(M-CH_3+HOH), 365, 339, 297, and 286. The peak corresponding to 24-methylene cycloartanol had a parent ion at *m/e* 440 and other peaks at 425 (M-CH_3), 422(M-HOH), 407(M-CH_3+HOH), 379, 353, 313, 300 and 297. These spectra are similar to those obtained by other workers for the same compounds.[4–6] The 4,4-dimethyl and 4α-methyl regions combined yielded cycloartenol (6·74%), 24-methylene cycloartanol (88·1%), and cyclocucalenol (5·18%).

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GLC analysis of the 4-desmethyl region showed the presence of β-sitosterol, stigmasterol, and campesterol, in the ratio 85·2:14·6:0·2. No effort was made to subject these 4-desmethyl sterols of *Cattleya* to GLC-MS analysis due to their ubiquitous nature in plants and the convincing evidence of their presence given by GLC.

It should be kept in mind that this examination entails two complex and different genera. Also the parts of the respective plants under examination differed greatly. The thin, reed-like stems of the *Arundina* examined have little physical resemblance to the bulky leaves and rhizomes representing the bulk of the *Cattleya* examined.

EXPERIMENTAL

Plants and sources. Flowering stems of *Arundina* (locally referred to as Bamboo Orchid or Kinta Weed in Malaya) were obtained in the market place of Kuala Lumpur. *Cattleya* plants, largely leaves and rhizomes, were obtained from the Missouri Botanical (Shaw's) Garden, St. Louis, Missouri.

Isolation and characterization of nonsaponifiable constituents. General procedures for extraction and isolation of the nonsaponifiable fraction were performed as previously described.[4] Column Chromatography, TLC, and preparative TLC was also performed as previously described.[4]

For GLC analyses, a Barber Colman model 5000 instrument was used with glass columns, 180 cm × 4 mm. For sterol analyses these columns were packed with 1% SE-30 and 3% OV-17 both on 100/120 Gas Chrom Q (Applied Science Labs., Inc.). Operating column temperature for 1% SE-30 and 3% OV-17 were 240° and 265° respectively. GLC analysis of the long chain alcohols was performed on a 1% QF-1 column at 170°. Orchid sterols retention times were compared with the retention times of reference sterols.

Combined GLC-MS was performed on a LKB model 9000 single focusing instrument. Sterol analyses was performed on a 240 cm glass column containing 1% SE-30 on 100/120 Gas Chrom Q. The MS were obtained under the following conditions: flash heater, 225°; column, 238°; molecular separator, 275°; ionizing energy, 70 eV; He flow, 30 ml/min. All spectra were compared with spectra obtained from authentic samples. GLC-MS analyses of the long chain alcohols was performed on a 3% QF-1 column. The mass spectra were obtained under the following conditions: flash heater, 240°; column, 200°; molecular separator, 225°; ion source, 290°; ionizing energy, 70 eV; He flow, 30 ml/min.

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