Fatty acids. Mixture from acidic part of the petrol extract; elution with C_6H_6 (Silica gel), and crystallization (Me_2O + MeOH) (m p , IR, methyl esters, GLC analysis) behenic acid (C_{22}), lignoceric acid (C_{24}), cerotic acid (C_{26}), montanic acid (C_{28}).

Oleanolic acid (m p , $[\alpha]_D$, IR, NMR, and *m/e* 456 (M^+), one COOH gp. and one ethylenic linkage). Acetate, methyl ester, methyl ester-acetate, lactone and ketone (m p and IR) From acidic part of the petrol extract after chromatography (Silica gel), elution with benzene–Et_2O (4 1), and crystallization (MeOH)

Oleanolic acid $\xrightarrow[\text{Jones' reagent}]{\text{oxidation}}$ oleanonic acid (Found· C, 79 45%, H, 10 60, $C_{30}H_{46}O_3$ required C, 79·29, H, 10 14%)

Other products Two triterpenic ketones (M^+, *m/e* 456 and 454), and an acid (M^+456) — (m p , $[\alpha]_D$, IR, UV and NMR) From petrol, C_6H_6 and MeOH extracts, chromatography and crystallization Belong to oleanolic acid series and are under investigation The acid appears to be 3-epi-oleanolic acid Ash of the plant material also has been analysed

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Key Word Index—Zataria multiflora, Labiatae, alkanes, phytosterols, triterpenes, fatty acids

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NEPENTHACEAE

STEROLS AND TRITERPENES OF THE PITCHER PLANT*

A S. WAN, R T. AEXEL, R B RAMSEY and H. J NICHOLAS

Department of Pharmaceutical Chemistry, University of Singapore, Singapore, the Institute of Medical Education and Research, and the Department of Biochemistry, St Louis University School of Medicine, St Louis, Missouri 63104, U S A

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Abstract—By means of column chromatography, TLC, GLC and GLC-mass spectrometry, the following sterols and triterpenes were found in free form in the pitcher plant, (*Nepenthes albomarginata*) cholesterol, campesterol, stigmasterol, sitosterol, isofuctosterol, β-amyrin and α-amyrin No 4α-methyl sterols were found in the free form The following sterols and triterpenes were identified in the esterified form cholesterol, campesterol, stigmasterol, sitosterol, isofucosterol, obtusifoliol, cycloeucalenol, citrostadienol, cycloartenol and 24-methylenecycloartanol Sitosterol was the major 4-desmethyl sterol in both the free and esterified fractions Previous examination of a glycoside fraction of *N sanguinea* yielded sitosterol as the major component

INTRODUCTION

OF THE various known carnivorous plants, those of the genus *Nepenthes* (Nepenthaceae) are the most prominent. To the best of our knowledge the nonsaponifiable constituents of

* Part II of the series, Part I of this series is Ref 1 "Nonsaponifiable constituents of Malaysian Plants". Requests for reprints should be addressed to. Dr H J Nicholas, Institute of Medical Education and Research, 1605 S 14th St St Louis, Missouri 63104, U S A

[1] A S WAN, R T AEXEL and H J NICHOLAS, *Phytochem* **10**, 2267 (1971)

this genus have not been examined. In a continuation of our studies on sterol and triterpene esters in plants, with the objective of determining the function of these compounds in plant tissue,[2,3] the sterol and triterpene constituents and the esters of two species of *Nepenthes* have been investigated. One species was identified as *N sanguinea*, and the other as *N. albomarginata*.

RESULTS

Examination of N sanguinea

The nonsaponifiable fraction from *N. sanguinea* was of sufficient quantity to subject the material to column chromatography on alumina and to attempt identification of the major constituents by m p , optical rotation, etc The material eluted with petrol was a deep red, mobile oil It appeared very complex by TLC and could not be fractionated further. The sterol fractions indicated the likelihood of the usual sitosterol–campesterol–stigmasterol mixture, but no well defined compound was obtained. However, from the highly polar fraction eluted with ethanol, a mixed glycoside of sitosterol–campesterol–stigmasterol was obtained, identified by m.p and hydrolysis to its constituents, which were verified by GLC Extensive analysis by GLC or GLC-mass spectrometry was not available when this material was examined.

Examination of N. albomarginata

The ethanol extract (70 g), of *N. albomarginata*, a black, viscous gum with a pungent odor, was chromatographed on alumina. Elution with petrol yielded a white crystalline hydrocarbon fraction. The first few benzene fractions yielded the ester fraction (661 mg). Fractions were subsequently eluted with benzene, diethyl ether (gradually increasing the percentage of diethyl ether in benzene), and finally ethanol. All fractions were monitored by TLC. Throughout the text TLC solvent systems will be designated as those found in Table 1. Fractions were combined according to their content as, 4-desmethyl- (653 mg), 4α-methyl- and 4,4-dimethyl sterols (326 mg) Further purification of each region was accomplished by preparative TLC [4]

TABLE 1 TLC SOLVENT SYSTEM

TLC Designation	Solvent	Ratio (vol)	R_f		
			4 desmethyl sterol	4α-methyl sterol	4,4 dimethyl sterol
Analytical	Trimethyl pentane–ethyl acetate–acetic acid	60 30 0 6	0 47	0 55	0 65
Preparative	Ethyl ether–benzene (3) Ethyl acetate–hexane (1)	12 88 1 3	0 44	0 54	0 64

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[4] R. RAHMAN, K SHARPLESS, T A SPENCER and R B CLAYTON, *J Biol Chem* **245**, 2667 (1970)

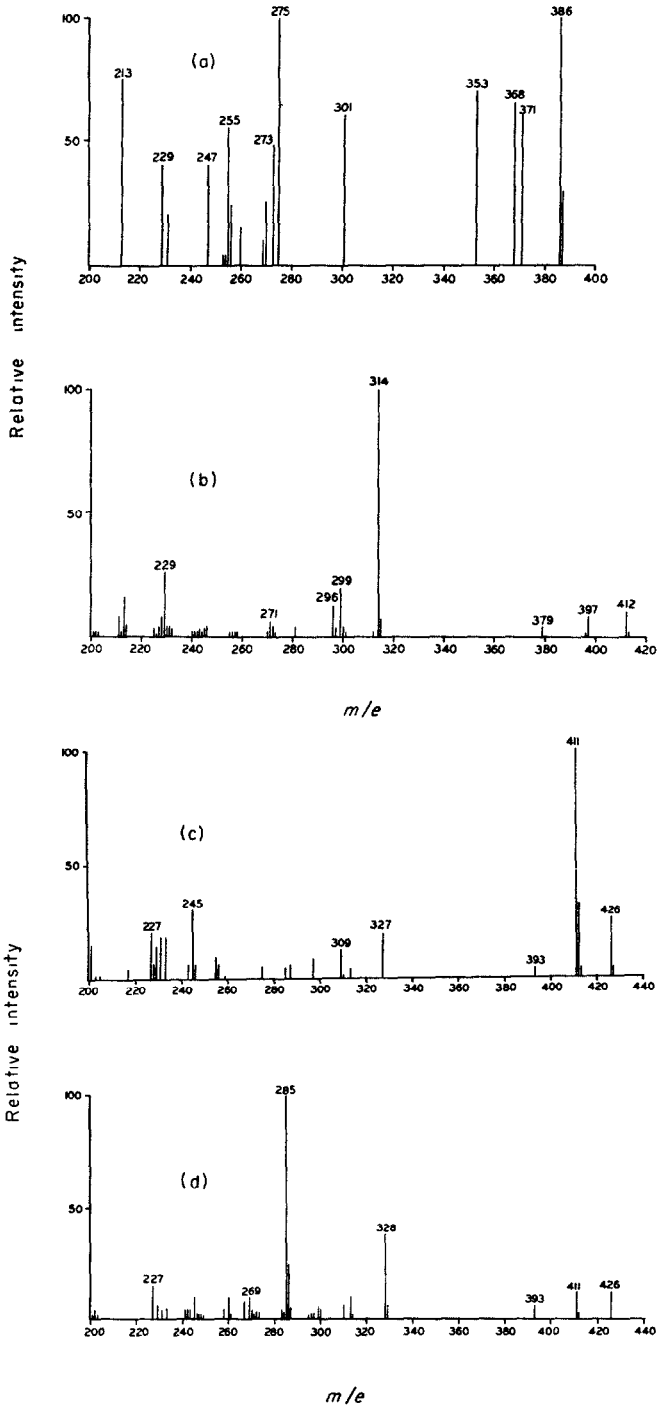

FIG 1 MASS SPECTRA OBTAINED BY GLC–MS ANALYSIS OF ISOLATED PRODUCTS FROM PITCHER PLANT
(a) cholesterol, (b) isofucosterol, (c) obtusifoliol, and (d) citrostadienol

Identification of 4-Desmethyl Sterols

Free GLC of the 4-desmethyl region yielded 5 major peaks. Peak 1 (1 per cent) had the same retention time (RT) as cholesterol. Combined GLC-MS of this substance (Fig. 1a) yielded a spectrum identical to a spectrum obtained for cholesterol under the same conditions and was similar to that obtained by other investigators.[5] The second (8 per cent), third (24 per cent), and fourth (52 per cent) peaks had the same RT on GLC as campesterol, stigmasterol and sitosterol respectively. GLC–MS of these compounds produced spectra identical to spectra obtained from authentic compounds and to previously published spectra [5,6] The fifth peak after conversion to an acetate had the same RT on GLC (3% OV-17) as standard isofucosterol acetate (Δ^5-avenasterol acetate). GLC-MS of this compound, as the free compound (Fig. 1b) was identical to the spectrum obtained by other investigators.[7] The fragment at *m/e* 314 is indicative of sterols having a $\Delta^{24(28)}$ double bond.[8]

Esterified The ester fraction yielded a 4-desmethyl sterol fraction whose content was identical to that of the free sterols. The distribution was as follows: cholesterol 1%, campesterol 8%, stigmasterol 8%, sitosterol 67%, and isofucosterol 16%.

Identification of 4α-Methyl Sterols

Free A careful analysis by GLC revealed that there were no major identifiable triterpenes in the 4α-methyl sterol region.

Esterified GLC analysis of the 4α-methyl region of the esterified sterols revealed three major peaks The first peak (45 per cent) had the same RT on GLC as obtusifoliol. GLC-MS of this compound (Fig. 1c) confirmed its identity as obtusifoliol. The presence of a C-24 methylene group was indicated by the fragment at *m/e* 327 [7] The spectrum was identical to that obtained by others.[9] The second peak (24 per cent) had the same RT on GLC as cycloeucalenol and the spectrum obtained from GLC-MS was identical to a spectrum of cycloeucalenol analyzed under the same conditions and spectra obtained by others [6,9,10] The third peak (31 per cent) had the same RT as citrostadienol (24-ethylidene lophenol) on GLC. GLC-MS produced a spectrum (Fig 1d) identical to a spectrum obtained from authentic citrostadienol and was similar to the spectra obtained by other investigators [11,12]

Identification of Tetracyclic and Pentacyclic Triterpenes

Free. A major portion of the crude free 4,4-dimethyl sterol fraction, obtained from column chromatography, was found to be a series of aliphatic alcohols. The first crystallization yielded white flocculent crystals, m.p. 77·5–79° GLC on 1% SE-30 and 3% OV-17 indicated the presence of the following alcohols· C_{24}, C_{26}, C_{28}, C_{30} and C_{32} A linear relationship was exhibited when the log of the RT was plotted against carbon number. The plot was superimposable with a plot obtained from a series of standard aliphatic alcohols

GLC analyses of the mother liquor indicated two major peaks The first peak (39 per

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[6] F F KNAPP and H J NICHOLAS, *Phytochem* **8**, 207 (1969)
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[10] H E AUDIER, R BEUGELMANS and B C. DAS, *Tetrahedron Letters* 4341 (1966)
[11] P BENVENISTE, L HIRTH and G OURISSON, *Phytochem* **5**, 31 (1966)
[12] B. L WILLIAMS, L. J GOAD and T W GOODWIN, *Phytochem* **6**, 1137 (1967)

cent) had the same RT as β-amyrin on GLC. GLC-MS produced a spectrum identical to a spectrum obtained from authentic β-amyrin The second peak (61 per cent) had the same RT as α-amyrin on GLC and GLC-MS produced a spectrum identical to a spectrum obtained from authentic α-amyrin. The spectra obtained from both β-amyrin and α-amyrin are similar to those obtained by previous investigators [13,14] We noted however, that while β-amyrin and α-amyrin had the same major fragmentation pattern, subtle differences existed in the spectra which enables distinction between them. The most notable difference is found in the relative intensities between *m/e* 189 and 203 In β-amyrin, *m/e* 189 had a relative intensity of 15% and *m/e* 203 had 45%, while in α-amyrin *m/e* 189 had 38% and *m/e* 203 had 44%.

Esterified GLC of this fraction yielded two major peaks and one very minor peak. The minor constituent (1 per cent) had the same RT as β-amyrin on GLC. A trace amount of α-amyrin was also detected in the mass spectrum of the second major peak GLC analysis of the first major peak (68 per cent) indicated that it had the same RT as cycloartenol and the second major peak (38 per cent) the same RT as 24-methylene cycloartanol. GLC–MS produced spectra identical to that obtained from authentic samples and were comparable to the fragmentation data reported by other investigators [6,10,12]

DISCUSSION

The composition of the free and esterified fractions of pitcher plant seems to be novel. No evidence has before been presented in the literature for such a clear cut distribution of unesterified pentacyclic and esterified tetracyclic triterpenes The identification of pentacyclic triterpenes in the same plant tissue with measurable amounts of tetracyclic triterpenes is rare, but not unknown. It is unique, however, in the case of the pitcher plant that all the 4α-methyl tetracyclic triterpenes present were in the esterified form. In contrast, the 4-desmethyl phytosterols were present in about the same distribution in both free and ester fractions

An extensive study of the distribution of free and esterified sterols and triterpenes in maize indicated a distribution completely opposite of that seen in the pitcher plant.[15,16] Free and esterified sterol and triterpene fractions from maize showed no significant differences of composition, what was present in the free was present in the corresponding ester fraction A similar situation was seen with *Strychnos nux-vomica*[17] and banana skins [2,3,6] There were no sterols present in the esterified form that were not present also in the free form Why such differences with regard to distribution between free and esterified forms should be seen in this plant is unknown, but must be related to the function of these two forms in the pitcher plant

EXPERIMENTAL

Nepenthes sanguinea, largely the pitcher and the immediate tendrils, was collected in the Cameron Highland area of Malaya on the slopes of Mt Gunong Asung *N albomarginata* consisting of the pitchers and the immediate adjacent tendrils was collected on Penang Island, Malaya The plants were carefully cleaned to remove any insects, dried, ground and extracted exhaustively with hot EtOH

Alumina (Merck acid-washed) was used in column chromatography Analytical TLC was performed on Silica Gel G (250 μ) and for preparative purposes, Silica Gel H (500 μ) was used In both systems, com-

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[16] R J KEMP and E I MERCER, *Biochem J* **110**, 119 (1968)
[17] F F KNAPP and H J NICHOLAS, *Mol Cryst Liq Cryst* **6**, 319 (1970)

pounds were revealed with either 20% $SbCl_5$ or 5% anisaldehyde in 95% ethanol–H_2SO_4, 19·1 v/v GLC was performed using a 3% OV-17 and a 1% SE-30 column, at 270° and 240° respectively Multiple analyses were made for each sample with reference samples being run before and after each sample GLC-MS was performed on a LKB Model 9000 single focusing gas chromatograph-mass spectrometer A 3% OV-17 column was used at a column temperature of 280°

A nonsaponifiable fraction was prepared from the ester fraction by refluxing it for 2 hr in a H_2O–benzene–EtOH (1/1/8, vol) solution containing 15% KOH, followed by extraction with Et_2O

Isolation of sterol glycoside mixture from *N sanguinea* The EtOH eluate (from an alumina column) yielded 300 mg of a solid melting about 290° to a dark red melt Repeated crystallization yielded a white solid, m.p 275–280°, $[\alpha]_D^{20°}$ − 44 08 (pyridine). Reported sitosteryl β-D-glycoside, m p 285°, with optical rotation at $[\alpha]_D^{20°}$ − 41 5 (pyridine) [18] (Calc for $C_{35}H_{60}$, C, 72 87, H, 10 45 (β-sitosteryl glycoside), Found. C, 72 23, H, 10 45%) Acetate formed from Ac_2O and pyridine (1 1 vol), m p 172–173°, $[\alpha]_D^{20°}$ 0° ($CHCl_3$), (Calc for $C_{43}H_{68}O_{10}$ C, 69 32, H, 9 20 (sitosterolin tetra-acetate) Found· C, 69 30, H, 9 12%)

Hydrolysis of the sterol glycoside from *N sanguinea* Refluxed (20 hr) in EtOH– HCl (5 1 vol). Mixture after dilution with H_2O, extraction with Et_2O and distillation of the latter yielded a white crystalline solid Repeated crystallization gave white crystals, m p 137–139°, acetate, m p 127–129° GLC of the free sterols indicated the presence of campesterol (5%), stigmasterol (10%), and sitosterol (85%)

Acknowledgements—We are indebted to Mr A Naqvi and Mr T Y Tok for their able assistance in this study and to Mr Chandapilla of the Rubber Research Institute of Malaysia for identification of the *Nepenthes* obtained from the Cameron Highlands area We are grateful to the following individuals for generous gifts of reference compounds· Drs J W Rowe (citrostadienol), F Knapp (28-isofucosterol acetate), A Gonzales (obtusifoliol) This work was supported by Grant GB 19113 from the National Science Foundation GLC–mass spectrometry was supported by NIH Grant AM-09992

[18] Elsevier's Encyclopedia of Organic Chemistry, *Sitosteryl Esters and Glycosides*, Series III, Vol 14, p 1820, Elsevier, Amsterdam (1954)

Key Word Index—Nepenthes albomarginata, *Nepenthes sanguinea*, Nepenthaceae, sterols, triterpenes

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PAPAVERACEAE

THE ALKALOIDS OF *ARGEMONE GRANDIFLORA*

M. H. BENN and R. E. MITCHELL

Department of Chemistry, The University, Calgary 44, Alberta, Canada

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Abstract—The major alkaloids of the above-ground parts of *Argemone grandiflora* Sweet subsp *grandiflora* were found to be berberine, α-allocryptopine, and protopine, (+)-laudanosine, (+)-codamine, (−)cheilanthifoline, corypalmine, sanguinarine, and chelerythine were identified as minor alkaloids This species is placed in Alliance IVa of the Stermitz classification, and appears to be a phylogenetically young member of the genus

INTRODUCTION

THE ALKALOIDS of *Argemone* species are of interest on account both of their pharmacological properties, and their potential use in the systematics of the genus. Thus the discovery[1] that 'epidemic dropsy' in India was caused by contamination of cooking oils with *Argemone mexicana* L seed-oil was followed by the demonstration[2] that the alkaloid sanguinarine, present in the seed-oil, increased intra-ocular pressure and caused dropsy and glaucoma in

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[2] S A HAKIM, *Br J Ophthal* **38**, 193 (1954)