on columns of Si gel, eluting with (a) C_6H_6 (b) C_6H_6–$CHCl_3$ (c) $CHCl_3$ (d) $CHCl_3$–Me_2CO (2:3) and (e) $CHCl_3$–Me_2CO–HOAc (4:6:1). The C_6H_6–$CHCl_3$ eluates yielded a small quantity of 3-chloroplumbagin, major quantity of plumbagin and mixtures of (i) 3-chloroplumbagin and plumbagin (ii) plumbagin. PZ 5, 3,3'-biplumbagin, isozeylinone (PZ 7) and chitranone, (iii) zeylinone (PZ 9) and elliptinone and (iv) sitosterol and droserone.

3-Chloroplumbagin and plumbagin were separated on a Si gel column using petrol (bp 40–60°)-C_6H_6 (4:1) as the eluent. The separation of mixtures of 3,3'-biplumbagin, isozeylinone and chitranone was difficult because of their close R_f values and the latter was separated on Si gel using C_6H_6–CCl_4 (17:3). Chitranone and mixtures of 3,3'-biplumbagin and isozeylinone were freed from impurities by extracting their ether solutions with 2% NaOH and 10% Na_2CO_3 respectively and regenerating them by acidification with dilute HCl, re-extracting into Et_2O and removing solvent under red pres. 3,3'-biplumbagin and isozeylinone have the same R_f values on TLC plates in a number of solvent systems; on recrystallization from MeOH the major component 3,3'-biplumbagin crystallized out. The mother liquor was evaporated under red pres and the NMR spectrum of the resulting solid showed the presence of 3,3'-biplumbagin and isozeylinone. Recrystallization of this solid 5× from MeOH gave pure isozeylinone. Eluents used for separation of (iii) zeylinone and elliptinone and (iv) sitosterol and droserone by Si gel column chromatography were EtOAc and $CHCl_3$ respectively. *3-Chloroplumbagin.* Ex petrol (bp 40–60°) and sublimed in vacuum, orange crystalline solid, mp 125° (150 mg). *Plumbagin.* Ex petrol (bp 60–80°), orange crystalline solid, mp 78° (68 g). *3,3'-biplumbagin.* Ex MeOH, orange crystalline solid, mp 214–6° (250 mg). *Isozeylinone (PZ 7).* Ex MeOH as orange crystalline solid, mp 192–4°, (5 mg); new compound, not yet characterized. *Chitranone (PZ 8).* Ex MeOH orange crystalline solid, mp 118–20° (81 mg). UV, $\lambda_{max}^{dioxane}$ (log ε); 257 nm (4·25), 437 nm (4·22); IR, ν_{max}^{KBr} 1660, 1640 cm^{-1}; NMR: 2·06 (*s*, CH_3); 2·22, (*d*, *J* 1·5 Hz, CH_3); 6·86, (*q*, *J* 1·5 Hz, vinylic –H); 7·16–7·42 (*q*, 1 Ar–H); 7·50–7·83 (*m*, 4 Ar–H); 11·96 (*s*, OH); 12·30 (*s*, OH); Mass spectrum (70 eV, Direct inlet, 200°):*m/e* (1%) 376(6), 375(25) 374(M^+) (100) 359(15) 346(10) 345(8) 331(7) 329(6) 317(6) 303(7) 187(M^{++}) (9) 121(10) 120(11) 92(19) 75(5) 64(6) 63(10) 39(9). *Zeylinone (PZ 9).* Ex CCl_4 yellow crystalline solid, mp 212–4° (200 mg), new compound, not yet characterized. *Elliptinone (PZ 10).* Ex $CHCl_3$ orange crystalline solid, mp above 300° (120 mg), MW: 374 (mass spectrum); NMR[5]: 2·22 (*d*, *J* 1·5 Hz, 2 and 2'–CH_3); 6·83 (*q*, *J* 1·5 Hz, 3 and 3'–H); 7·71 (*s*, 7,7',8, and 8'–H); 12·48, (*s*, 5 and 5'–OH). *Droserone (PZ 11).* Ex MeOH and sublimed in vacuum, orange crystalline solid, mp 175° (125 mg). *Sitosterol.* Ex MeOH mp 135–6° (1 g).

The identity of 3-chloroplumbagin, plumbagin, 3,3'-biplumbagin, elliptinone, droserone and sitosterol was established by mmp, IR and co-TLC with authentic samples.

Chitranone dimethyl ether was purified by column chromatography over Si gel and recrystallized from $CHCl_3$-petrol (bp 60–80°) (1:3), brownish-yellow crystalline solid, mp 198°. Found: C, 71·70, H, 4·85%; $C_{24}H_{18}O_6$ requires C, 71·63, H, 4·51%; MW: 402 (mass spectrum); IR, ν_{max}^{KBr} 1660 cm^{-1}, 1630 cm^{-1}; NMR: 1·98 (*s*, 2–CH_3); 2·20 (*d*, *J* 1·5 Hz, 2'–CH_3); 6·80 (*q*, *J* 1·5 Hz, 3'–H); 3·74 (*s*, 5'–OMe); 3·99 (*s*, 5–OCH_3); 7·38–8·10 (*m*, 5 Ar–H of which 7·81, *d*, (*J* 8 Hz) 7'–H; and 8·02, *d*, *J* 8 Hz, 8'–H can be identified).

Acknowledgements—We thank Dr. J. Tamas, Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest-11 and Dr. K. G. Das, National Chemical Laboratory, Poona for mass spectral data, Dr. S. Natori, National Institute of Hygienic Sciences, Tokyo and Prof. R. H. Thomson, University of Aberdeen, for samples of elliptinone and Prof. F. Bohlmann, Technical University, Berlin for the 100 Mc NMR spectrum of elliptinone and Dr. N. Ramayya for identification of plant material.

REFERENCES

1. Sidhu, G. S. and Sankaram, A. V. B. (1971) *Tetrahedron Letters*, 2385.
2. Bowie, J. H., Cameron, D. W. and Williams, D. H. (1965) *J. Am. Chem. Soc.* **87**, 5094.
3. Sidhu, G. S. and Pardhasaradhi, M. (1967) *Tetrahedron Letters*, 4263.
4. Yoshihira, K., Tezuka, M., Takahashi, C. and Natori, S. (1971) *Chem. Pharm. Bull.* **19**, 851.
5. Yoshihira, K., Tezuka, M., Kanchanapee, P. and Natori, S. (1971) *Chem. Pharm. Bull.* **19**, 2271.

Phytochemistry, 1976, Vol 15, pp. 238–240. Pergamon Press. Printed in England

LIPIDS, STEROLS, AND A PIPERIDINE ALKALOID FROM *PROSOPIS SPICIGERA* LEAVES

KENNETH JEWERS,* MARTIN J. NAGLER,* KARIMULLAH A. ZIRVI† and FEHMIDA AMIR†

*Tropical Products Institute, 56/62 Gray's Inn Road, London, WC1X 8LU, England; †P.C.S.I.R. Laboratories, Peshawar University, Peshawar, Pakistan

(*Revised received* 25 *June* 1975)

Key Word Index—*Prosopis spicigera*; Leguminosae, constituents, piperidine alkaloid, spicigerine, lipids, sterol glycosides, sterols.

Recently we reported [1] the isolation of a new piperidine alkaloid, spicigerine, from the leaves of *Prosopis spicigera*. We wish now to describe in detail the methods used for its isolation, and in addition report on some of the other constituents present in the leaves.

A hexane extract of the dried leaves was concentrated to a small volume, allowed to stand overnight, and the solid which precipitated out was filtered off. The solid was chromatographed over alumina; this afforded two fractions. The fraction eluted with petrol was analysed by GC and was found to consist of aliphatic hydrocarbons (1%), esters (92%) and alcohols (7%). The hydrocarbon fraction consisted entirely of hentriacontane, and the alcohol fraction was a mixture of octacosan-1-ol (8·8%) and triacontan-1-ol (91·2%). The composition of the ester fraction is given in Table 1.

Table 1. The aliphatic esters of *P. spicigera* leaves

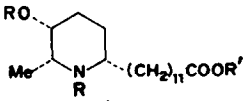
Carbon no.	%	Carbon no.	%	Carbon no.	%
C_{46}	0·5	C_{55}	2·3	C_{60}	18·2
C_{48}	2·1	C_{56}	9·6	C_{61}	1·7
C_{50}	6·8	C_{57}	4·6	C_{62}	1·9
C_{51}	0·8	C_{58}	17·1	C_{64}	0·4
C_{53}	1·5	C_{59}	5·9		
C_{54}	15·0				

Methanolysis of the ester fraction gave a mixture of methyl esters of aliphatic acids, and aliphatic alcohols. GC analysis of these fractions furnished the results shown in Table 2.

Elution of the column with mixtures of petrol and C_6H_6 gave a solid mp 288°. Trituration of this solid with dichloromethane gave soluble and insoluble fractions. GC analysis of the soluble portion showed that it consisted of cholesterol (4%), campesterol (12%), sitosterol (60%) and stigmasterol (24%). These findings were confirmed by MS studies.

The IR and MS of the insoluble fraction suggested that it consisted of a mixture of sterol glycosides. The NMR of the acetate showed resonances for four acetoxy groups, indicating that it was a mixture of sterol monoglycosides. This was confirmed by acid hydrolysis and GC analysis of the products, which showed the presence of cholesterol, campesterol, sitosterol and stigmasterol.

The leaves after extraction with hexane were exhaustively extracted with EtOH, and the EtOH extract was separated into basic and non-basic fractions. Chromatography of the basic fraction over alumina afforded spicigerine (**1a**), $C_{18}H_{35}$-NO_3 (M^+ *m/e* 313·2627), mp 196°. The IR spectrum of spicigerine showed strong absorptions at 1580 and 1650 cm^{-1} which indicated that the molecule was an amino acid. High resolution MS studies showed that spicigerine had the structure (**1a**). The base peak, *m/e* 114, ($C_6H_{12}NO$) arose by elimination of the side chain with charge retention on the piperidyl moiety.



(1a) R, R' = H
(1b) R = Ac, R' = Me

Table 2. Composition of the methyl esters and alcohols (as acetates) derived from the ester fraction of *P. spicigera* leaves

Carbon no.	Contents % Acids (as Me ester)	Alcohol
C_{16}	2·0	—
C_{18}	0·8	—
C_{20}	1·8	—
C_{22}	6·3	—
C_{24}	10·1	—
C_{26}	5·5	1·5
C_{28}	10·9	23·9
C_{30}	41·2	72·0
C_{32}	21·0*	2·5

*Most of this component did not seem to be a normal C_{32} ester.

Strong ions corresponding to M-15 and M-1 showed that a methyl group and a hydrogen atom were attached to the carbon atoms α- to the nitrogen of the piperidine ring. The ions observed at *m/e* 296, 268, 73 and 60 confirmed the presence of a carboxyl group in spicigerine, and a series of ions from *m/e* 128 to 254 at 14 amu intervals showed [3] that the largest alkyl group attached to the piperidine ring was unbranched and consisted of eleven methylene groups terminated by a carboxyl group. Loss of water from the molecular ion and the base peak indicated that piperidyl moiety contained a hydroxyl group.

Methylation of spicigerine with diazomethane followed by acetylation with acetic anhydride furnished methyl diacetylspicigerine (**1b**), $C_{23}H_{41}NO_5$ (M^+, *m/e* 411), $[\alpha]_D^{25}$ −6·3 (*c*, 0·63; $CDCl_3$) as an oil. Strong absorption in the IR spectrum at 1720, 1615 and 1250 cm^{-1} indicated the presence of *O*-acetyl, *N*-acetyl, and carboxymethyl groups; these were confirmed by three proton singlets at δ2·04, 2·09 and 3·64 respectively in the NMR spectrum. The NMR spectrum also showed the presence of a polymethylene side chain (*bs*, δ 1·25), and a proton on a carbon atom bearing an acetoxy group (1H, *m*, δ4·80). The MS of methyl diacetylspicigerine was consistent with the structure (**1b**) assigned to the molecule [1].

ORD studies on methyl diacetylspicigerine have shown that the molecule has a negative plain curve. Cassine and a number of 2-alkyl piperidines have been reported [3] to have negative plain curves, and the lactones carpaine and azimine have been shown to have [4] negative Cotton effects associated with the $n \rightarrow \pi^*$ transition of the ester carbonyl group. These results suggest that spicigerine has the absolute configuration (**1a**), but confirmation of this will have to await the isolation of a larger quantity of the alkaloid.

EXPERIMENTAL

Isolation of the constituents of the hexane extract. 1·5 kg of dried powdered leaves of *P. spicigera* was exhaustively extracted with hexane. The hexane extract was concentrated, left at room temp. overnight, and the solid which had separated out was filtered off. Solid was absorbed onto a column of aluminium oxide which was successively eluted with light petrol and mixtures of light petrol and C_6H_6. Light petrol eluted a wax consisting of aliphatic hydrocarbons, alcohols and esters, the composition of which was determined by GLC using f.i.d. and a stainless steel column (1 m × 3 mm) of 1·5% Dexsil 300 on 80–100 mesh Chromosorb W, the temp. was programmed from 125°–400° at 3°/min, and He flow rate was 60 ml/min. Methanolysis of the ester fraction gave a mixture of methyl esters of fatty acids and aliphatic alcohols which were analysed after acetylation by GLC using the above column and programming the temp. from 125° to 300° at 3°/min. Elution of the alumina column with light petrol–C_6H_6 (1:1) gave a solid, mp 288°, which could be separated into soluble and insoluble fractions by trituration with C_6H_6. GLC analysis of the soluble portion using a glass column (1·5 m × 6 mm o.d.) of 2% SE30 on 100–120 mesh Gas-Chrom Q at an oven temp. of 260° and N_2 flow rate of 50 ml/min. showed that it consisted of cholesterol–campesterol–sitosterol (1:3:15). The MS and IR of the insoluble portion suggested that it was a mixture of sterol glycosides. Acid hydrolysis using 2N H_2SO_4 gave a mixture of cholesterol, campesterol, sitosterol and stigmasterol.

Isolation of spicigerine. The defatted leaves were exhaustively extracted with EtOH. The insoluble portion of the EtOH extract was filtered off, the filtrate was evaporated to dryness and the residue was dissolved in 5% acetic acid. The acid

soluble portion was basified with NH_4OH, then extracted with $CHCl_3$, and the resultant extracts dried (Na_2SO_4). Evaporation of the $CHCl_3$ gave a semi-solid, which was absorbed onto a column of neutral aluminium oxide and the column was developed successively with C_6H_6, mixtures of C_6H_6 and EtOAc and EtOH. Benzene–EtOAc (1:1) furnished spicigerine, mp 196°, (M^+, *m/e* 313·2617; Calc. for $C_{18}H_{35}NO_3$: M^+, *m/e* 313·233.)

Acknowledgements—We wish to thank Mr. F. Cottee, The Wellcome Research Laboratories, for high resolution mass spectral data, and Dr. A. P. Tulloch, Prairie Regional Laboratory, National Research Council, Canada, for the GC analysis of the wax.

REFERENCES

1. Jewers, K., Nagler, M. J., Zirvi, K. A., Amir, F. and Cottee, F. H. (1974) *Pahlavi Med. J.* **5**, 1.
2. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1967) *Mass Spectrometry of Organic Compounds*, Holden Day, San Francisco.
3. Lythgoe D. and Vernengo, M. J. (1967) *Tetrahedron Letters* 1133.
4. Smalberger, T. M., Rall, G. J. H. and de Waal, H. L. (1968) *Tetrahedron* **24**, 6417.

Phytochemistry, 1976, Vol 15, pp 240–241 Pergamon Press Printed in England

ACRIDON-ALKALOID-GLUKOSIDE AUS *RUTA GRAVEOLENS**

J. Reisch, Zs. Rozsa, K. Szendreit, I. Novák und E. Minker

Institut für Pharmazeutische Chemie der Westfälischen Wilhelms-Universität Münster/W., Pharmakognostisches Institut und Pharmakologisches Institut der Medizinischen Universität, Szeged (Ungarn)

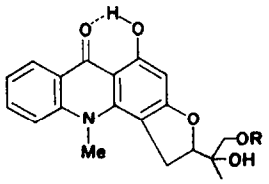
(*Received* 19 *June* 1975)

Key Word Index—*Ruta graveolens*; Rutaceae; acridone alkaloids; glucosidic acridones; gravacridontriol; gravacridondiol glucoside; gravacridontriol glucoside.

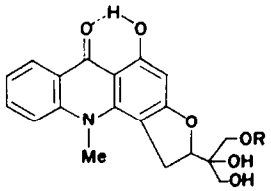
Alkaloid–Glykoside sind insgesamt gesehen bisher äußerst selten als Pflanzeninhaltsstoffe beschrieben worden. Allein die Sterin-Alkaloide liegen zum überwiegenden Teil glykosidisch gebunden in der Pflanze vor [1, 2]. Erstmalig konnten nun Acridon-Glykoside in *Ruta graveolens* aufgefunden werden.

Acridon–Alkaloide sind sehr schwache Basen und trotz mehr oder weniger starker Hydroxylierung am Kern und an der isoprenoiden Partialstruktur, schlecht wasserlöslich. Infolgedessen lassen sie sich aus der Pflanze oder deren Methanolextrakt mit Petroläther und Benzol leicht extrahieren.

Beim Aufarbeiten des Methanolextraktes der Wurzeln von *Ruta graveolens* fiel die stark gelbe Färbung der wäßrigen Phase nach der Benzol- und $CHCl_3$-Ausschüttelung auf. Die gelbe Komponente ließ sich mit ÄtOAc extrahieren. Wie die DC-Analyse ergab, enthält dieser Extrakt neben Flavonoid–Glykosiden auch Substanzen, die in ihrem analytischen Verhalten den Acridon–Alkaloiden ähneln [3, 4]. Durch Chromatographie an Polyamid konnte ein kristallines Gemisch von zwei Glykosiden isoliert werden, dessen Komponenten sich auf diese Weise nicht voneinander trennen ließen.



(1) R = H (Gravacridondiol)
(2) R = Glc



(3) R = H (Gravacridontriol)
(4) R = Glc

Bei der sauren Hydrolyse des Gemisches entstanden neben Glukose zwei Acridon–Alkaloide, die als Gravacridondiol (**1**) [5] und Gravacridontriol (**2**) identifiziert wurden. Beide Aglyka kommen in der Wurzel zusätzlich in freier Form vor. Gravacridondiol wurde von uns bereits früher beschrieben [5]. Gravacridontriol konnte jetzt aus der gleichen Fraktion, in der sich auch die beiden Glukoside befanden, isoliert werden.

Gravacridontriol **2** erwies sich durch sein Hauptfragment-Ion *m/e* 266 als 2-substituiertes 5-Hydroxy-6-oxo-11-methyl-1,2,6,11-tetrahydro-[2,3-*c*]-furoacridin; in die gleiche Richtung weisen seine NMR-Daten die weitgehend mit **1** [5] und dem Gravacidonolchlorin [6] übereinstimmen. Infolge der beiden quartären Kohlenstoffatome zeigen die jeweils benachbarten Zentren, d.h. die $ArC\underline{H}_2$- und die Methin-Gruppe in gleicher Weise wie eine der beiden nicht äquivalenten Methylen-Gruppen ein komplexeres Aufspaltungsmuster, ein Befund, auf den bei ähnlich angeordneten Strukturelementen bereits verschiedentlich hingewiesen wurde [7–10]. Die betreffenden Signale sind bei **3** zusätzlich von anderen Resonanzen überlagert, wodurch ihre weitere Analyse beeinträchtigt wird.

Wie aus dem Verhältnis der Extinktionen der Aglyka und der Glukoside bei 272, 5 nm sowie dem Verhältnis

Färbung, infolgedessen sollte das Acridon-Alkaloid über eine seiner alkoholischen Gruppen mit der Glukose verbunden sein. Die Klärung der Frage, ob die Verknüpfung über eine primäre oder die tertiäre alkoholische Gruppe erfolgt, ergibt sich aus dem IR-Spektrum des nach Umsetzung mit Ac_2O in Pyridin erhaltenen Produktes. Eine deutlich erkennbare OH-Bande bei 3300/cm weist darauf hin, daß in den Acetaten noch eine freie alkoholische OH-Gruppe enthalten ist. Da unter den genannten Bedingungen die tertiäre OH-Gruppe am trägsten reagiert,

* LV. Mitt.: "Studien auf dem Gebiet der Naturstoffchemie" LIV. Mitt.: J. Reisch, M. Gombos, K. Szendrei u. I. Novák Phytochemistry (im Druck).

† Derzeitige Adresse: Scientific and Technical Section, United Nations Narcotic Division, Genève.