Synthesis of N$^\alpha$-*CM*-L-*lysine* (**2**). N^α-CM-L-lysine was prepared by carboxymethylation of N^ε-Z-L-lysine (2.00 g, 7.1 mM) according to a similar procedure to that for N^ε-CM-lysine described above [1]. Mp 237–240° (decomp.). (Found: C, 46.17; H, 7.83; N, 13.30. Calcd for $C_8H_{16}O_4N_2 \cdot 1/4H_2O$: C, 46.03; H, 7.97; N, 13.42%.

REFERENCES

1. Wadman, S. K., De Bree, P. K., Van Sprang, F. J., Kamerling, J. P., Haverkamp, J. and Vliegenthart, J. F. G. (1975) *Clin. Chim. Acta* **59**, 313.
2. Schwyzer, R., Costopanagiotis, A. and Sieber, P. (1963) *Helv. Chim. Acta* **46**, 870.
3. Itoh, M., Hagiwara, D. and Kamiya, T. (1977) *Bull. Chem. Soc. Jpn* **50**, 718.

Phytochemistry, 1979, Vol. 18, pp. 662–663. ©Pergamon Press Ltd. Printed in England. 0031-9422/79/0401-0662 $02.00/0

MUCILAGE IN CALLUS CULTURES OF HIGHER PLANTS

C. K. KOKATE* and S. S. RADWAN†

Federal Centre for Lipid Research, Piusallee 68, D-4400 Münster, Germany‡

(*Received* 24 *August* 1978)

Key Word Index—Callus cultures; mucilage; monosaccharides; galactose; mannose.

INTRODUCTION

Mucilage commonly occurs in higher plants [1, 2] but, so far, this class of natural products has not received any attention by workers studying plant cell cultures. Since mucilage finds wide industrial application [3], it was considered worthwhile to investigate callus cultures of some medicinally and economically important plants for their mucilage contents and for the composition of constituent monosaccharides in the mucilage. The results of this study are summarized in the present communication.

RESULTS AND DISCUSSION

The data presented in Table 1 indicate that callus cultures of higher plants are relatively rich in mucilage, which commonly makes up 8–10% of the dry wt. In callus cultures of *Trigonella foenum-graecum*, 21.2% mucilage is found. The seeds and 3-week-old seedlings of *Trigonella foenum-graecum* contain 26.3 and 10.8% mucilage, respectively.

Galactose and mannose appear to be the common constituent monosaccharides in mucilage of callus cultures. In mucilage from cultures of *Catharanthus roseus*, glucose instead of galactose, is detected and in mucilage from *Nicotiana glauca*, only galactose is present. In the case of *T. foenum-graecum* and *Glycine max*, a striking similarity between the qualitive and quantitative composition of mucilage in callus cultures (Table 1) and mucilage in organs of the intact plant [4, 5] is observed.

* Permanent address: The Bombay College of Pharmacy, Kalina, Bombay-29, India.

† Permanent address: Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt.

‡ Address for correspondence and reprints (Prof. S. S. Radwan).

Table 1. Mucilage contents* of callus cultures and monosaccharides of hydrolysates

Callus culture	Mucilage content	Monosaccharides (ratio)
Trigonella foenum-graecum	21.2	Galactose:mannose (1:1·4)
Catharanthus roseus	8.4	Glucose:mannose (1·2:1)
Cicer arietinum	8.9	Galactose:mannose (1·5:1)
Glycine max	8.1	Galactose:mannose (1:1·2)
Nicotiana glauca	9.4	Galactose

* Values are expressed in % dry wt.

EXPERIMENTAL

Callus cultures of *T. foenum-graecum* and *C. roseus* were established from sterile seedlings on MS medium [6]. Cultures of *Cicer arietinum*, *G. max* and *N. glauca* were kindly provided by Prof. W. Barz, University of Münster, Münster, Germany. Eight-week-old cultures were dried *in vacuo* at 60° for 12 hr. Mucilage was extracted with 5% aq. HOAc, precipitated from the conc soln by excess EtOH, purified, dried to constant wt and determined gravimetrically [7]. Mucilage was hydrolysed by heating in a sealed tube with 2 N H_2SO_4 at 100° for 1 hr. The soln was cooled, neutralized with $BaCO_3$, passed through Amberlite IR-120 and concd *in vacuo* [8]. The monosaccharides formed by hydrolysis were identified by TLC together with authentic samples on Kieselguhr G plates impregnated with Pi buffer (pH 5) using n-BuOH–Me_2CO–Pi buffer, pH 5 (4:5:1) as the developing solvent [9]. The monosaccharides were also analysed qualitatively and quantitatively by GLC of their TMSi derivatives [10] using a glass column (2 m × 6 mm) packed with Chromosorb W, 80–100 mesh, coated with 15% Carbowax 20 M. Column temp. was 220°, N_2 was carrier gas. The proportions of the constituent monosaccharides were determined from their rel. peak areas.

Acknowledgement—This investigation was supported by the Deutscher Akademischer Austauschdienst', D-5300 Bonn-Bad Godesberg 1, Germany.

REFERENCES

1. Smith, F. and Montgomery, R. (1959) *The Chemistry of Plant Gums and Mucilages*. Am. Chem. Soc. Monograph Ser. No. 141, p. 4. Reinhold, New York.
2. Anderson, E. (1949) *Ind. Eng. Chem.* **41**, 2887.
3. Smith, F. and Montgomery, R. (1959) *The Chemistry of Plant Gums and Mucilages*. Am. Chem. Soc. Monograph Ser. No. 141, p. 501. Reinhold, New York.
4. Andrews, P., Hough, L. and Jones, J. K. N. (1952) *J. Chem. Soc. III*, 2744.
5. Whistler, R. L. and Saarnio, J. (1957) *J. Am. Chem. Soc.* **79**, 6055.
6. Murashige, T. and Skoog, F. (1962) *Physiol. Plant.* **15**, 473.
7. Dutta, A. B. (1961) *J. A.O.A.C.* **44**, 640.
8. Smith, F. and Montgomery, R. (1959) *The Chemistry of Plant Gums and Mucilages*. Am. Chem. Soc. Monograph Ser. No. 141, p. 98. Reinhold, New York.
9. Lewis, B. A. and Smith, F. (1967) in *Dünnschicht-Chromatographie* (Stahl, E., ed.) p. 776. Springer, Berlin.
10. Sweeley, C. C., Bentley, R., Makita, M. and Wells, W. W. (1973) *J. Am. Chem. Soc.* **85**, 2498.

Phytochemistry, 1979, Vol. 18, pp. 663–664. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0401-0663 $02.00/0

ON THE NATURAL OCCURRENCE OF 15α-TIGLINOYLOXY-KAUR-16-EN-19-OIC ACID

THEREZINHA C. B. TOMASSINI* and MARIA ELISA OLIVEIRA MATOS

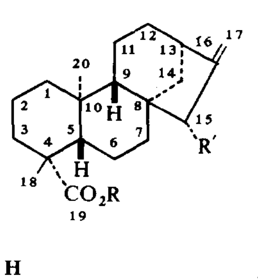
Núcleo de Pesquisas de Productos Naturais, Centro de Ciências da Saúde, Bloco H, Universidade Federal do Rio de Janeiro, ZC-32-Brazil

(*Received* 2 *August* 1978)

Key Word Index—*Wedelia scaberrima*; Compositae; kaur-16-en-19-oic acid; molluscicide; 15α-tiglinoyloxy-kaur-16-en-19-oic acid; miracidicide; ^{13}C NMR.

The observation that a hexane extract of *Wedelia scaberrima* Benth. showed activity against snails prompted a chemical examination in an effort to identify the active constituents. Kaur-16-en-19-oic acid **1** and 15α-tiglinoyloxy-kaur-16-en-19-oic acid **2** could be isolated. Working on *Wedelia* species, Bohlmann *et al.* [1] prepared the methyl ester of compound **2** from a complex acid mixture.

Compound **2** has a tigloyl group CH_3—CH=C(CH_3)-COO^- confirmed by MS (base peak m/e = 83, 100%, $C_4H_7CO^+$) and by ^{1}H NMR spectrum which exhibited signals at 6.84 (*m*, 1H) and 1.83 (*s*, 6H' s)δ. Double resonance studies showed that irradiation of the methyl signals resulted in the collapse of the vinyl proton multiplet to a singlet and irradiation of the vinyl proton signal resulted in the opening of the singlet methyl protons to a double singlet signal. The hydrolysis product of **2** was 15α-hydroxy-kaur-16-en-19-oic acid $C_{20}H_{30}O_3$ (*m/e*, 318); mp 232–234° (lit. 228–230°) [2].



1 R = R' = H
2 R = H; R' = tigl. = $-O_2C^{1'}-C^{2'}=C^{3'}$(H)(CH_3 4'), CH_3 5'
3 R = H; R' = OH
R = Me; R' = tigl.

Table 1. ^{13}C-NMR spectrum of **2***

C-1	40.80 *t*	C-14	37.67 *t*
C-2	18.55 *t*	C-15	83.35 *d*
C-3	37.87 *t*	C-16	156.48 *s*
C-4	43.98 *s*	C-17	110.67 *t*
C-5	56.96 *d*	C-18	29.02 *q*
C-6	20.95 *t*	C-19	185.81 *s*
C-7	35.14 *t*	C-20	15.88 *q*
C-8	48.08 *s*	C-1'	169.16 *s*
C-9	53.28 *d*	C-2'	129.80 *s*
C-10	40.09 *s*	C-3'	137.93 *d*
C-11	19.13 *t*	C-4'	14.45 *q*
C-12	32.86 *t*	C-5'	12.24 *q*
C-13	42.83 *d*		

* Run in $CDCl_3$.

The ^{13}C NMR spectrum (Table 1) of the tigloyl ester confirmed its structural identity. The absorption at 185.81 ppm as a singlet, proved the presence of the other two oxygens in the molecule in total agreement with the MS (m/e = 400, 7%, $C_{25}H_{36}O_4$). Treatment of **2** with diazomethane gave the methyl ester **4** whose ^{1}H NMR spectrum exhibited the signal of a methyl group at 3.64δ, confirming the presence of the carboxylic acid in **2**.

Pakraski *et al.* isolated the 15α-angeloyloxy-kàur-16-en-19-oic [3] from *Enhydra fluctuans* Lour. In an attempt to synthesize the ester, the isomeric tiglate was obtained.

Tests performed with the isolated compounds showed **1** to be molluscicidal (against *Biomphalaria glabata*) and **2** miracidicidal (against miracidia of *Schistosoma mansoni*).

* Author to whom correspondence should be addressed.