yielded substance E (1·22 g) as light citron coloured needles from EtOH–$CHCl_3$. The EtOAc eluates were conc and crystallised from EtOH to yield substance F (0·16 g). Substance A, mp 125°, $[\alpha]_D$ + 46°, $C_{31}H_{52}O$. IR ν_{max} cm^{-1}: 3450 (OH), 1658, 889 (C=CH_2), 1000 (cyclopropane). NMR ($CDCl_3$): δ 0·30 and 0·58 (2H, *d*, *J* 5Hz, cyclopropyl H): 0·80–1·05 (18H, 6Me), 1·63 (3H, *s*, Me–C=C), 3·3 (1H, *m*, CHOH) and 4·7 (2H, C=CH_2). MS *m/e*: 440 (M^+) 425, 422, 407, 379, 353, 315, 300, 297, 185. Monoacetate, mp 120°, $C_{33}H_{54}O_2$, M^+ at *m/e* 482; monobenzoate. mp 193°, $C_{38}H_{56}O_2$, M^+ at *m/e* 544. It was oxidised to a keto derivative, mp 114°, $C_{31}H_{50}O$, M^+ at *m/e* 438, having a six membered ring carbonyl function (1710 cm^{-1}) and yielded formaldehyde on ozonolysis. It was identified as cyclolaudenol [8]. Substance B, mp 138°, $[\alpha]_D$-40°, acetyl derivative mp 128°, was confirmed as sitosterol (mmp). Substance C, mp 278°, $[\alpha]_D$-46°, gave a positive Fiegel's test. It was hydrolysed with 6 N HCl which yielded sitosterol and glucose. It was, therefore, established as sitosterol-β-D-glucoside. Substance D, mp 309–10°, $C_{30}H_{48}O_3$, ν_{max} cm^{-1}: 3380 (OH), 2900, 2840, 1700 (COOH), 1390, 833 (–CH=C–). Monoacetate, mp 265°; methyl ester, mp 198–200°; methyl ester acetate, mp 218–20°. It was identified as oleanolic acid (mmp, TLC, IR, NMR).

Substance E (queretaroic (30) caffeate). mp 230–2°, $[\alpha]_D$ + 152°, R_f 0·2 ($CHCl_3$–MeOH 96:4). IR ν_{max} cm^{-1}: 3380 (OH), 2900, 2850, 1710 (COOH), 1380, 840 (C=CH). UV λ_{max} nm: 217, 250, 302 and 333 (log ε 4·279, 4·185, 4·247, 4·346). NMR: δ 0·76, 0·85 (6H each, *s*, 4 Me), 0·9, 1·03 (3H each, *s*, 2 Me), 3·2 (1H, *m*, CH–OH), 4·5 (2H, *s*, CH_2O–), 5·73 (1H, *m*, C=CH), 6·5, 7·85 (2H, *d*, *J* 16Hz, *trans* CH=CH), 7·3–7·45 (3H, Ar). MS *m/e*: 634 (M^+). (Found: C, 73·71; H, 8·46. $C_{39}H_{54}O_7$ requires C, 73·82; H, 8·52%).

Acetyl derivative, mp 225–8°. IR ν_{max} cm^{-1}: 2900, 2850, 1790 (ArOAc), 1735 (OAc), 1710 (COOH), 1380, 840. UV λ_{max} nm: 222, 280 (log ϵ 3·96, 4·09). NMR: δ 0·78–1·0 (18H, *s*, 6 Me), 2·05 (3H, *s*, OCOMe), 2·33 (6H, *s*, 2 ArOCO Me), 4·35 (2H, *m*, CH_2O), 4·5 (1H, *m*, CHOAc), 5·73 (1H, *m*, C=CH), 6·5, 7·82 (2H, *d*, *J* 16Hz, CH=CH), 7·3–7·45 (3H, Ar). (Found: C, 70·89; H, 7·81. $C_{45}H_{60}O_{10}$ requires C, 71·00; H, 7·89%).

Methylester acetate. Reaction of the acetyl derivative with ethereal CH_2N_2 gave colourless needles from MeOH, mp 212–7°. IR ν_{max} cm^{-1}: 2970, 2860, 1785 (ArOAc), 1730 (OAc), 1735 (COOMe), 1380, 1685, 1250, 1205, 1176, 1111, 903, 845. NMR: δ 0·72–1·05 (18H, *s*, 6 Me), 2·05 (3H, *s*, OCO Me), 2·25 (6H, *s*, 2 × ArOCO Me), 3·60 (3H, *s*, COOMe), 4·3 (2H, *d*, *J* 7Hz, CH_2O–), 4·5 (1H, *m*, CHOAc), 5·6 (1H, *m*, C=CH), 6·5, 7·82 (2H, *d*, *J* 16Hz, CH=CH), 7·25, 7·55 (3H, Ar). MS *m/e*: 774 (M^+).

Alkaline hydrolysis of substance E. A soln of substance E (500 mg) in 10% alcoholic NaOH (30 ml) was kept for 48 hr at room temp. The reaction mixture was acidified and extracted with $CHCl_3$ followed by EtOAc which yielded two products, E-A1 and E-A2, respectively. E-A1 crystallised from MeOH–$CHCl_3$, mp 318–20°. It gave a violet colour with $SOCl_2$ and a yellow colour with tetranitromethane. IR ν_{max} cm^{-1}: 3480, 2950, 2860, 1710 (COOH), 1395, 833, 1475, 1050. NMR: δ 0·8–1·08 (18H, 6 Me), 3·3 (1H, *m*, CHOH), 3·86 (2H, *d*, *J* 7Hz, CH_2O), 5·78 (1H, *m*, C=CH), MS *m/e*: 472 (M^+).

EA1 acetate. mp 295–9°. IR ν_{max} cm^{-1}: 2950, 2870, 1750, 1710, 1377, 1480, 1250, 835. NMR: δ 0·73–0·95 (18H, 6 Me), 2·05 (6H, *s*, 2 OCOMe), 4·15 (2H, CH_2OAc), 4·53 (1H, *m*, CHOAc), 5·61 (1H, *m*, C=CH). MS *m/e*: 496 (M^+ – 60).

EA1 methylester acetate. mp 210°. IR ν_{max} cm^{-1}: 2970, 2850, 1735 (OCOMe), 1730 (COOMe). 1375, 1450, 1250, 908, 835. NMR: δ 2·02, 2·05 (3H each, *s*, OCOMe), 3·65 (3H, *s*, COOMe), 4·14 (2H, –CH_2OAc), 4·5 (1H, *m*, CHOAc), 5·62 (1H, *m*, C=CH). MS *m/e*: 510 (M^+ – 60). Component E-A2 mp 209° decomp., pale yellow, gave green colour with ferric chloride. IR ν_{max} cm^{-1}: 3400, 1655 (unsaturated COOH), 1610, 1535, 1450 (conjugated phenyl), 860, 822 (1,2,4-trisubstituted phenyl), 1215, 980 and 785. UV λ_{max} nm: 298 (*Sh*), 326, *NMR* (acetone d_6): δ 6·23, 7·50 (1H each, *d*, *J* 16Hz, –CH=CH), 6·67–7·20 (3H, *m*, Ar). MS *m/e*: 180 (M^+), 173, 162, 77, 63 and 51. *Acetyl derivative,* mp 198°. IR ν_{max} cm^{-1}: 1754, 1205 (ArOAc).

Methyl ester. mp 179°. IR ν_{max} cm^{-1}: 2835, 1695, 1639, 1613, 1525, 845, 822, 1460, 1429, 980 and 774. MS *m/e*: 208 (M^+), 193, 161, 133, 119, 91, 77. Substance F, mp 281°, $C_{30}H_{48}O_3$. Monoacetate, mp 244–5°; methyl ester, mp 170°. It was confirmed as ursolic acid (TLC, IR, NMR and MS).

Acknowledgements—The authors thank Messrs. R. K. Mukerji, B. B. P. Srivastava, R. K. Singh for IR, NMR and MS data respectively and Mr. E. Samson for technical assistance.

REFERENCES

1. Anderson, L. A. P. (1968) *J.S. African Chem. Inst.* **21**, 91.
2. Anderson, L. A. P. and Koekemoer, J. M. (1968) *J.S. African Chem. Inst.* **21**, 155.
3. Anderson, L. A. P. and Koekemoer, J. M. (1969) *J.S. African Chem. Inst.* **22**, 191, 5119.
4. Koekemoer, J. M., Anderson, L. A. P. and Pachler, K. G. R. (1970) *J.S. African Chem. Inst.* **23**, 146.
5. Koekemoer, J. M., Anderson, L. A. P. and Pachler, K. G. R. (1971) *J.S. African Chem. Inst.* **24**, 75.
6. Dhar, M. L., Dhawan, B. N., Prasad, C. R., Rastogi, R. P., Singh, K. K. and Tandon, J. S. (1974) *Indian J. Exp. Biol.* **12**, 512.
7. Rangaswami, S. and Sarangan, S. (1969) *Tetrahedron* **25**, 3701.
8. Henry, J. A. Irvine, D. S. and Spring, F. S. (1955) *J. Chem. Soc.* 1607.

Phytochemistry, 1976, Vol. 15, pp. 432–433 Pergamon Press. Printed in England

THE ISOLATION FROM *SARRACENIA FLAVA* AND PARTIAL SYNTHESIS OF BETULINALDEHYDE

J. BHATTACHARYYA, U. KOKPOL and D. H. MILES*

Department of Chemistry, Mississippi State University, Mississippi State, MS, 39762, U.S.A.

Key Word Index—*Sarracenia flava*; Sarraceniaceae; betulinaldehyde; partial synthesis.

We recently reported the isolation of a new iridoid, sarracenin, from the roots of the insectivorous plant *Sarracenia flava*. We also confirmed the antitumor activity in the roots of this plant and isolated the known antitumor agents lupeol and betulin along with sitosterol, α-amyrin and large quantities of betulinic acid. The

* To whom inquiries should be sent.

benzene extract of the aerial parts of this plant showed the highest antileukemic activity (% T/C 150 at a dose level of 50 mg/kg against p. 388 lymphocytic leukemia in BDF_1 mice) [1–3]. We now wish to report the isolation of betulinaldehyde (1) and traces of betulin (2) from the most active fraction of the benzene extract.

The benzene extract, after removal of a large quantity of betulinic acid (3), was subjected to column chromatography. Fractions which retained the highest antileukemic activity upon repeated column chromatography yielded betulinaldehyde and sarracenin along with traces of betulin. Although betulin is a known antitumor agent, the amount of this compound present in the most active fraction does not justify the relatively high activity. Sarracenin was also found to be inactive in the preliminary cytotoxicity tests. This seems to indicate that betulinaldehyde is the compound responsible for most of the activity in the potent fraction.

A relatively large amount of betulinaldehyde is required for detailed tests of its probable antileukemic activity. Methyl betulinate [4] was converted to the tetrahydropyrano ether (THP-ether) (4). Vitride reduction [5] of (4) furnished the THP-ether of betulin which was smoothly oxidized by active manganese dioxide [6] to the THP-ether of betulinaldehyde (5). Betulinaldehyde (1) was then obtained by hydrolysis of this ether in 2·8 molar methanolic *p*-toluene sulfonic acid. The over all yield was approximately 40%.

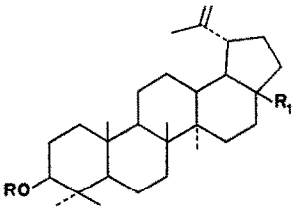
Although betulinaldehyde is a known compound [8,9], it has been found in only a few plants [4] and usually in very small quantities, whereas, the related compounds, betulinic acid, betulin and lupeol, are present in many plants in larger quantities. The relative lack of stability of betulinadehyde might preclude its isolation when betulinic acid, betulin and lupeol are also present. The partial synthesis from betulinic acid, provides an easy access to the aldehyde.

EXPERIMENTAL

Mp's are uncorrected. Si gel G (Woelm) was used for column chromatography. Chromatography was monitored by TLC on Si gel G plates with Me_2CO–C_6H_6–$CHCl_3$ (1:2:17) as developer. All new compounds gave satisfactory elemental analysis.

Isolation of betulinaldehyde (1). Dried and ground plant material (5 kg) was extracted with hexane for 24 hr (Soxhlet). Residual plant material was then extracted with C_6H_6 for 36 hr. C_6H_6 extract was conc to 2 l. and cooled. Betulinic acid was removed by filtration and the filtrate further concentrated and chromatographed over a column (74 × 4 cm) and 500 ml fractions were eluted successively with C_6H_6–hexane 1:1 (6 l.), C_6H_6 (4 l.), C_6H_6–$CHCl_3$ 9:1 (30 l.) and C_6H_6–$CHCl_3$ 4:1 (20 l.. Fraction A). Fraction A (5 g) was rechromatographed on a column (40 × 3·5 cm) and 500 ml fractions were eluted successively with benzene (4 l.), C_6H_6–$CHCl_3$ 4:1 (10 l.), C_6H_6–$CHCl_3$ 1:1 (9 l.: Fraction B). Fraction B (1·5 g) was rechromatographed over a column (30 × 2·5 cm) and eluted successively with C_6H_6–$CHCl_3$ 9:1 in 200 ml fractions. Fractions 5 through 9 on evaporation and crystallization from hexane gave transparent needles, mp 188–190°, *m/e* 440 (M^+); ν_{max} (Nujol) 3340 (OH), 1750 (–CHO) and 895 cm^{-1}. The compound gave a precipitate with 2,4-dinitrophenylhydrazine reagent and its other spectral properties are identical with those reported [4] for betulinaldehyde. A direct comparison was not possible because an authentic sample was not available.

Isolation of Sarracenin. Fractions 10 through 17 of the chromatography of Fraction B were rechromatographed on a column (45 × 3 cm) and 500 ml fraction were collected successively with hexane, hexane–C_6H_6 1:1 through 1:9 (24 l.), C_6H_6 (25 l.), C_6H_6–$CHCl_3$ 9:1 (8 l.) and C_6H_6–$CHCl_3$ 4:1 (1 l.). The last 12 l. eluate after concentration and crystallization from hexane gave transparent needles, mp 127–128°, $[\alpha]_D^{27}$–68·8° ($CHCl_3$), identical with sarracenin in all respects [1].



(1) R = H, R_1 = CHO

(2) R = H, R_1 = CH_2OH

(3) R = H, R_1 = CO_2H

(4) R =  R_1 = CO_2Me

(5) R =  R_1 = CHO

THP-ether of methyl betulinate (4). Methyl betulinate (9·4 g) from betulinic acid [4] was refluxed in CCl_4 (50 ml) with dihydropyran (1·68 g) and *p*-toluene sulfonic acid monohydrate (1·9 g) for 2 hr. Removal of the solvent *in vacuo* gave a residue which was treated with cold H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed (sat. $NaHCO_3$ and H_2O), dried (Na_2SO_4) and evaporated. The residue was crystallized from hexane in white needles (8·35 g, 75%), mp 198–200°, *mze* 554 (M^+), ν_{max} (KBr) 2900–1720, 1640, 885 cm^{-1}. NMR ($CDCl_3$), δ 0·86 (6Hs) 0·93 (3H, *s*), 1·02 (3H, *s*), 1·05 (3·05, *s*), 1·80 (3H, *s*), 4·80 (1H, *m*), and 4·96 (1H, *m*).

THP-ether of betulin. Vitride reduction of THP-ether of methyl betinate [5]. THP-ether of methyl betulinate (8·34 g) in Et_2O (200 ml) was treated with a 70% C_6H_6 soln of sodium bis-(2-methoxyethoxy) aluminum hydride (22·5 g) dropwise with stirring and the mixture refluxed for 1 hr. The excess reagent was decomposed, ether removed and the residue extracted with $CHCl_3$, washed (H_2O) and dried (Na_2SO_4). Removal of $CHCl_3$ gave a white solid which was crystallized from benzene in white needles, (5·14 g, 65%), mp 218–20°, ν_{max} (KBr) 3300(OH), 2800, 1640 and 875 cm^{-1}; MS at *m/e* 526 (M^+), 495, 425, 385, 313, 284, 256 and 85.

THP-ether of betulinaldehyde (5). THP-ether of betulin (5·0 g) in methylene chloride (500 ml) and active MnO_2 [6] was stirred at room temp. for 10 hr. The oxidation was monitered by TLC. After all of the starting material disappeared, MnO_2 was removed and the filtrate evaporated. Residue was crystallized from C_6H_6–hexane (4·48 g, 90%), mp 164–167°. The compound gave a precipitate with 2,4-dinitrophenylhydrazine. ν_{max} (KBr) 2890, 1710, 1640, 890, and 875 cm^{-1}. MS at *m/e* 524(M^+), 496, 439, 423, 395, 189, and 85.

Betulinaldehyde (1): *hydrolysis of the THP ether of betulinaldehyde* [7]. THP ether of betulinaldehyde (4·0 g) was dissolved in MeOH containing 2·8 mMol of *p*-toluene sulfonic acid and the soln stirred at room temp. for 2 hr. MeOH was removed *in vacuo* and the residue treated with cold H_2O and extracted with $CHCl_3$. The organic layer was washed (sat. $NaHCO_3$ and H_2O), dried and concentrated. The residue was crystallized from C_6H_6–hexane (3·00, 90%). ν_{max} (KBr) 3300, 2890, 1700, 1640, 885 cm^{-1}, MS at *m/e* 440 (M^+), 411, 256, 236, 207, and 189. Calcd. for $C_{30}H_{48}O_2$, MW 440. The compound was found to be identical with the natural betulinaldehyde (*vide supra*) in NMR, and IR comparisons.

Acknowledgements—Supported by grant number 3-ROI-CA-13268, National Cancer Institute, National Institutes of

Health, Bethesda, Md. 20014. We also wish to thank Dr. Sidney McDaniel for collection of plant material.

REFERENCES

1. Miles, D. H., Kopol, U., Bhattacharyya, J., Atwood, J. L., Stone, K. E., Bryson, T. A. and Wilson, C. (1975) *J. Am. Chem. Soc.*
2. Miles, D. H., Kokpol, U., Zalkow, L. H., Steindel, S. J. and Nabors, J. (1974) *J. Pharm. Sci.* **63**, 613.
3. Miles, D. H. and Kokpol, U. (1975) *J. Pharm. Sci.* (In press).
4. Pakrashi, S. C., Bhattacharyya, J., Mookerjee, S. and Vorgbrüggen, H. (1968) *Phytochemistry* **7**, 461 (and refs. cited therein).
5. Cerny, M., Malek, J., Capka, M. and Chvalovsky, V. (1969) *Coll. Czech. Chem. Commun.* **34**, 1025.
6. Allenburrow, J., Cameron, A. F. B., Chapman, J. H., Evans, R. M., Hems, B. A., Jansen, A. B. A. and Walker, T. (1952) *J. Chem. Soc.* 1094.
7. Corey, E. J., Achiwa, K. and Katzenellenbogen, J. A. (1969) *J. Am. Chem. Soc.* **91**, 4318.
8. (a) Ruzicka, L. and Rey, E. (1941) *Helv. Chim. Acta* **24**, 529. (b) Ruzicka, L. and Brenner, M. (1939) *Helv. Chim. Acta* **22**, 1523.
9. Aplin, R. T., Halshall, T. G. and Norin, T. (1963) *J. Am. Chem. Soc.* **85**, 3273.

Phytochemistry, 1976, Vol. 15, pp. 433–435. Pergamon Press. Printed in England.

STEREOCHEMISTRY OF MOLLUGOGENOL-A AND MOLLUGOGENOL-E, THE TRITERPENOID SAPOGENOLS FROM *MOLLUGO HIRTA*

MANASH KUMAR CHOUDHURY and PARUL CHAKRABARTI

Department of Chemistry, Bose Institute, Calcutta 9, India

(*Received* 29 *March* 1975)

Key Word Index—*Mollugo hirta*; Ficoidaceae; pentacyclic triterpene; stereochemistry of the hydroxyisopropyl side chain; mollugogenol-A; mollugogenol-E.

In an earlier communication [1], the structure of mollugogenol-A, a new triterpenoid sapogenin isolated from *Mollugo hirta*, was proposed as 3β,6α,16β,22-tetrahydroxyisohopane (**1**) on the basis of the spectral analysis and chemical reactions. Mollugogenol-A was finally degraded to zeorininone (**5**), a degradation product of zeorin (**3**), a triterpene of the hopane series. However this work did not determine the sterochemistry of the hydroxyisopropyl side chain at C-21.

Hydrogenolysis of the side chain of mollugogenol-A in the presence of hydrogen and PtO_2 in acetic acid was found to be very slow and furnished mainly, unreacted product together with a small amount of the saturated compound (**8**). Mollugogenol-A therefore behaved like a member of the isohopane rather than of the hopane series [2]. However, there was no conclusive chemical proof regarding the stereochemistry of the hydroxyisopropyl side chain. With a view to obtaining more direct evidence, the problem has been re-investigated and it has been possible to convert mollugogenol-A to 6-ketoisohopane (**11**) which has clearly established the β-orientation (isohopane) [3] of the side chain. Thus the structure of mollugogenol-A has now been finally established as (**1**) and consequently that of mollugogenol-E [4] as (**4**), the oxidation product of which was correlated with the oxidation product of mollugogenol-A. Mollugogenol-A fur-