78·31; H, 11·35%. Calc. for $C_{30}H_{52}O_3$, C, 78·20; H, 11·38%). IR spectra showed no CO. Acetylation (at room temperature and at 130°) gave a diacetate (without any trace of triacetate) m.p. 230–231° $[\alpha]_D^{27}$ + 32° (found: C, 74·89; H, 10·42; acetyl, 15·59, calc. for $C_{34}H_{56}O_5$, C, 74·95; H, 10·36; acetyl, 15·72%). Stability of this diacetate towards CrO_3 proved that the OH-group must be tertiary one i.e. at C – 13. The triol diacetate was dehydrated with BF_3 in dry benzene [9], purified on a $AgNO_3$ impregnated silica gel column, to give a major fraction which on crystallization ($CHCl_3$–MeOH) yielded a pure compound m.p. 295–296° $[\alpha]_D^{27}$ + 70·2°. Although the compound gave yellow colour with $C(NO_3)_4$, its IR spectra showed no absorption for a trisubstituted double bond. UV210 (ϵ = 5650) 215 (4520) and 220 nm (3650), were indicative of a tetrasubstituted double bond [10]. On oxidation with CrO_3 in HOAc [11] a conjugated ketone was obtained, m.p. 290° $[\alpha]_D^{27}$ + 65°. IR 1690 cm^{-1}, and UV, at 242 nm (ϵ = 13520) without any absorption for vinylic proton in NMR. Thus the position of double bond introduced by dehydration of triol-diacetate is most likely to be at C 13(18). In view of the evidence outlined above the structure of the new hydroxylactone is suggested to be 3β-hydroxy-lupane-13β-28-lactone (**1**).

Acknowledgement—The authors thank Dr U. R. Ghatak, Indian Association for the Cultivation of Science, Calcutta-32 for NMR spectra.

REFERENCES

1. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956). *Glossary of Indian Medicinal plants*. Council of Scientific and Industrial Research, (India), p. 97.
2. Bhattacharjee, S. R. and Chatterjee, A. (1962) *J. Ind. Chem. Soc.* **39**, 276.
3. Lebreton Philippe and Bouchez Marie-Paule (1967) *Phytochemistry* **6**, 1601.
4. Robertson, A., Soliman, G. and Owen, E. C. (1939) *J. Chem. Soc.* 1267.
5. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
6. Cheung, H. T. and Williamson, D. G. (1969) *Tetrahedron* **25**, 119.
7. Marx, M., Leclercq, J., Turch, B. and Djerassi, C. (1967) *J. Org. Chem.* **32**, 3150.
8. Adhikary, S. P., Lawrie, W. and McLean, J. (1970) *J. Chem. Soc.* (C), 1030.
9. Djerassi, C., Farkas, E., Liu, L. H. and Thomas, G. H. (1955) *J. Am. Chem. Soc.* **77**, 5330.
10. Halsall, T. G. (1951) *Chem. Ind. London*, 867.
11. Djerassi, C. and Mills, J. S. (1958) *J. Am. Chem. Soc.* **80**, 1236.

Phytochemistry, 1975, Vol. 14, pp. 1448–1450. Pergamon Press. Printed in England.

ASEBOTIN AND ITS AGLUCONE FROM THREE SPECIES OF *RHODODENDRON*

TOM J. MABRY AND MASAYUKI SAKAKIBARA

Cell Research Institute and Department of Botany, University of Texas at Austin, Austin, TX 78712, U.S.A.

and

BRUCE KING

Department of Botany, University of Georgia, Athens, GA 30601, U.S.A.

(*Received* 10 *July* 1974)

Key Word Index—*Rhododendron canescens*, *R. nudiflorum*, *R. roseum* and *R. calendulaceum*; Ericaceae; dihydrochalcones; asebotin; 2′,6′,4-trihydroxy-4-methoxydihydrochalcone.

In the course of a biochemical systematic investigation of *Rhododendron* (Ericaceae), we isolated two dihydrochalcones, one of which was not previously reported from Nature. In a recent review, Bohm [1] noted that there are only 13 naturally occurring dihydrochalcones including phloridzin (2′,4′,6′,4-tetrahydroxydihydrochalcone 2′-*O*-glucoside) and asebotin (the 4′-*O*-methyl ether of

phloridzin) from *Kalmia* and *Pieris* (Ericaceae). From this same family we now report asebotin (**1**) and its aglucone (**2**) from three species of *Rhododendron* (*R. canescens*, *R. nudiflorum* and *R. roseum*; the glucoside was also detected in *R. calendulaceum*). This is the first report of the aglucone 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone as a natural product.

The glucoside–aglucone relationship of **1** and **2** was established when acid hydrolysis of **1** gave glucose and **2**. The UV spectra of both compounds in MeOH (λ_{max} 283–284 nm and ~325 sh) indicated that the *B*-ring was not conjugated with the C_4-carbonyl group (i.e. either a dihydrochalcone, dihydroflavonol or flavanone) and the NMR spectra of the TMS ethers of both compounds established the dihydrochalcone structure for both substances (typical [2] broadened singlet for the four protons on the α- and β- carbon atoms at δ2·85 in CCl_4 with some splitting in C_6D_6 supporting the presence of a $-CH_2CH_2-$ system). The NMR spectrum of the TMS derivatives of the glucoside exhibited two doublets at δ5·93 and 6·13 (*J* 2Hz) for the two *A*-ring protons indicating oxygen substituents at the 2′, 4′ and 6′-positions. In addition, the NMR spectra of the TMS ethers of both **1** and **2** had a singlet typical for an aromatic methoxyl group (δ3·70 for **1**; 3·67 for **2**) and since the UV spectrum of the glucoside (in contrast the aglucone gave a bathochromic shift with a band I peak at 362 nm) was essentially unchanged upon the addition of NaOMe to the methanol solution of **1**, both the methoxyl and the *O*-glucosyl groups must occupy some combination of the 2′ and 4′-positions. (The 6′-hydroxyl group is hydrogen bonded to the C_4 carbonyl group). Moreover, since the *A*-ring protons show the typical meta coupling in the NMR spectrum of the TMS ether of the glucoside but are equivalent in the spectrum of the TMS ether of the aglucone, the methoxyl group must be at the 4′-position, a conclusion supported by the large benzene induced shift (+0·34 ppm) of the methoxyl signal relative to its chemical shift in CCl_4 [3]. Therefore, the glucosyl moiety must be attached at the 2′-position and hence the glucoside is asebotin.

The MS of the aglucone further supported the structure assignment: while no parent ion was observed, a base peak appeared at *m/e* 107 for a fragment ion $(C_7H_7O)^{+\cdot}$ from a *B*-ring and an intense peak (50% abundancy based on the base peak) at *m/e* 167 for a fragment ion $(C_8H_7O_4)^{+\cdot}$ supporting the presence of two hydroxyl groups and a methoxyl group in the *A*-ring. Other intense peaks were at *m/e* 120 (30%) for a fragment ion $(C_8H_8O)^{+\cdot}$, *m/e* 140 (17%) for a fragment ion $C_7H_8O_3)^{+\cdot}$, and *m/e* 181 (7·5%) for a fragment ion $(M-C_7H_7O)^{+\cdot}$.

EXPERIMENTAL

All the *Rhododendron* leaf material was collected in the Callaway Gardens, Pine Mountain, Georgia, with the assistance of the Director of Horticulture of the Gardens, Mr. Fred Galle, who identified all the plant material. Herbarium specimens are deposited in the Univ. of Georgia Herbarium (GA) under the following collection numbers: *R. canescens* (Michx.) Sweet, King 6; *R. nudiflorum* (L.) Torr., King 27; *R. roseum* (Loisel.) Rehder, King 43; and *R. calendulaceum* (Michx.) Torr., King 12. In each case, the air-dried leaf material was extracted with 80% aq MeOH. Compounds **1** and **2** were isolated by polyamide column chromatography. The polyclar polyamide column was packed in MeOH and after addition of the extract in 80% aq. MeOH to the top of the column elution was effected by a modified Egger's solvent ($CHCl_3$–MeOH–MEK–Me_2CO, 20:10:5:1). The first compound to be eluted from the column was the aglycone (**2**) followed by the glucoside (**1**); the several flavonol glycosides present in the extract were eluted after the dihydrochalcones. The UV [4], NMR [4] and MS [5] spectra were recorded using standard procedures.

2′,6′,4-Trihydroxy-4′-methoxy-dihydrochalcone (**2**): mp 162–3°; (color on paper under UV with and without NH_3: purple): UV (nm): λ_{max}(MeOH): 224, 284, 327 sh. λ_{max}(NaOMe): 238, 293, 362; λ_{max}($AlCl_3$): 221, 308, 370; λ_{max}($AlCl_3$/HCl): 205 sh, 221, 306, 368; λ_{max} (NaOAc): 217 sh, 283, 324 sh, λ_{max} (NaOAc/H_3BO_3): 219 sh, 284, 320 sh.; NMR (δ) of TMS ether in CCl_4: 0·20 (*s*, 27H, 3 × OTMS), 2·87 (*s*, 4H, α- and β- methylene groups), 3·67 (*s*, 3H, -OMe), 5·87 (*s*, 2H, 3′- and 5′-H), 6·57 (*d*, *J* 8 Hz, 2H, 3- and 5-H), 6·97 (*d*, *J* 8Hz, 2H, 2- and 6-H). NMR (δ) of TMS ether in C_6D_6: 0·17 (*s*, 27H, 3 × OTMS), 2·9–3·3 (multiplet containing sharp peaks at δ3·13 and 3·23, 4H, α- and β- methylenes), 3·33 (*s*, 3H, -OMe), 6·17 (*s*, 2H, 3′- and 5′-H), 6·77 (*d*, *J* 8Hz, 2H, 3- and 5-H), 7·02 (*dd* due to long range coupling, *J* 2, 8Hz, 2H, 2- and 6-H). MS data in text.

Asebotin (**1**): mp 135–6°; (color on paper under UV: purple; with NH_3, lighter purple; UV (nm): λ_{max}(MeOH): 224, 283, 320 sh; λ_{max}(NaOMe): 227, 282, 320 sh; λ_{max}($AlCl_3$): 220, 308, 359 sh; λ_{max}($AlCl_3$ HCl): 220, 304, 359 sh; λ_{max}(NaOAc): 221, 282, 316 sh; λ_{max} (NaOAc H_3BO_3): 220, 282, 316 sh. NMR (δ) of TMS ether in CCl_4: 2·88 (*s*, 4H, α-and β-methylenes), 2·8–3·8 (*m*, 6H, glucosyl protons), 3·70 (*s*, 3H, -OMe), 4·92 (*m*, 1H, glucosyl anomeric H), 5·93 (*d*, *J* 2Hz, 1H 3′- or 5′-H) 6·13 (*d*, *J* 2Hz, 1H 5′- or 3′-H), 6·60 (*d*, *J* 8Hz, 2H, 3- and 5-H), 7·00 (*d*, *J* 8Hz, 2H, 2- and 6-H).

Acknowledgements—This work was supported at The University of Texas by the Robert A. Welch Foundation (Grant F-130) and the National Science Foundation (Grant BMS 71-01088) and at the University of Georgia by the National Science Foundation (Grant GB-35480) and by an Ida Cason Callaway Foundation Grant for dissertational research.

REFERENCES

1. Bohm, B. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. J. eds) Chapman and Hall, London (in press).
2. Star, A. E. and Mabry, T. J. (1971) *Phytochemistry* **10**, 2817.
3. Rodriguez, E., Carman, N. J. and Mabry, T. J. (1972) *Phytochemistry* **11**, 409.
4. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer-Verlag, Heidelberg.
5. Mabry, T. J. and Markham, K. R., (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. J. eds.) Chapman and Hall, London (in press).

Phytochemistry, 1975, Vol. 14, p. 1450. Pergamon Press. Printed in England.

MICROMERIC ACID FROM *SALVIA HORMINUM*

A. ULUBELEN
Faculty of Pharmacy, University of Istanbul, Turkey
and
C. H. BRIESKORN
Institute of Pharmacy and Food Chemistry, University of Würzburg, Germany

(*Received* 26 *June* 1974)

Key Word Index—*Salvia horminum*; Labiatae; micromeric acid; sitosterol; triterpenic and steroidal alcohols.

INTRODUCTION

The presence of triterpenes in some *Salvia* species has long been known. Ursolic and oleanolic acids were isolated from *S. officinalis* [1–5], *S. triloba* [6–8] and *S. apiana* [9]. Other triterpenes were obtained from *S. officinalis* [10–12] and *S. apiana* [9] and a new triterpene, anagadiol, was found in *S. broussonetti* [13].

*S. horminum**, which grows in Turkey, has not been previously investigated. From the upper ground parts of the plant ursolic, oleanolic and micromeric acids were isolated. Although micromeric, ursolic and oleanolic acids have been found together in other plants of the Labiatae, micromeric acid is reported for the first time in *Salvia*. The acid was first isolated from *Micromeria bentham* [14] and later, was found in the leaves of *Rosmarinus officinalis* [15].

EXPERIMENTAL

Salvia horminum was collected from the Mediterranean coast of Turkey. The dried and powdered plant was extracted successively with light petrol and $CHCl_3$. The petrol extract was fractioned on neutral Al_2O_3 (activity III) giving five triterpenoid and steroidal compounds one of which was sitosterol (m.m.p., IR).

The $CHCl_3$ extract gave a main mixed band (R_f 0·62) by preparative TLC (silica gel G with $CHCl_3$:EtOH, 9:1) not separable by crystallization or argentized TLC. NMR and MS indicated that the band was a mixture. Separation of the mixed acetylated methyl esters by GLC (2% XE60, on WHP (Aw-DMCS) with 30 ml/min N_2 at 250°) gave three peaks R_t equivalent to the derivatives of oleanolic, ursolic and micromeric acids.

* The plant was identified by Prof. Dr. A. Baytop (Istanbul). A voucher sample ISTE 8032 is deposited in the Herbarium of Faculty of Pharmacy, University of Istanbul.

REFERENCES

1. Brieskorn, C. H. and Schlumprecht, L. (1951) *Arch. Pharm.* **284**, 239.
2. Brieskorn, C. H. and Eberhardt, K. H. (1952) *Arch. Pharm.* **285**, 124.
3. Brieskorn, C. H. and Weskamp, R. (1960) *Pharm. Acta Helv.* **35**, 183.
4. Kurt, H. and Devetak, Z. (1956) *Bull. Soc. Chim. Rep. Populaire* (Bosnie and Herzegovine) **5**, 15; *Chem. Abstr.* **51**, 13320e.
5. Pourrat, H. and Le Men, J. (1953) *Anal. Pharm. Franc.* **11**, 191.
6. Theodossiou, Ph. (1959) *Trav. Soc. Pharm. Montpellier*, **19**, 172; *Chem Abstr.* **54**, 25082g.
7. Brieskorn, C. H., Klinger, H. and Polonius, W. (1961) *Arch. Pharm.* **294**, 389.
8. Ulubelen, A., Oztürk, S. and Isildatici, S. (1968) *J. Pharm. Sci.* **6**, 1037.
9. Pettit, G. R., Klinger, H., Jorgensen, O. N. and Occolowitz, J. (1966) *Phytochemistry* **5**, 301.
10. Brieskorn, C. H. and Grossletter, A. (1964) *Arch. Pharm.* **297**, 456.
11. Brieskorn, C. H. and Polonius, H. (1962) *Pharmazie* **17**, 705.
12. Leiner, U. (1954) Dissertation, Istanbul.
13. Gonzales, A. G., Breton, J. J. and Fraga, B. M. (1971) *Chem. Commun.* 567.
14. Berrera, B. J., Breton Funes, J. L., Martin de la Fuente, G. and Gonzales, G. A. (1968) *Anal. Quim.* **64**, 175; *Chem. Abstr.* **69**, 19326g.
15. Brieskorn, C. H. and Zweyrohn, G. (1970) *Pharmazie* **25**, 488.