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STRUCTURE OF THE β-ORCINOL DEPSIDONES, CONNORSTICTIC AND CONSALAZINIC ACIDS

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Key Word Index—*Pertusaria pseudocorallina*; *Parmotrema subisidiosum*; lichens; connorstictic; consalazinic.

Abstract—The structures of connorstictic acid from *Pertusaria pseudocorallina* and consalazinic acid from *Parmotrema subisidiosum* were determined.

INTRODUCTION

It has been frequently observed [1] that the β-orcinol depsidones, norstictic and salazinic acids are often accompanied by varying amounts of two minor metabolites known as connorstictic and consalazinic acid, respectively. We are unaware of any report of the occurrence of these metabolites unaccompanied by their co-metabolites and indeed the relatively low concentrations at which these two compounds are normally found have hitherto hindered their isolation and identification. We now report the isolation and identification of connorstictic acid from the crustose lichen *Pertusaria pseudocorallina* (Sw.) Arn. where it occurs in reasonably high concentration with norstictic acid. Consalazinic acids have been reported (M. E. Hale, personal communication) in *Parmotrema subisidiosum* (Mull. Arg.) Hale, a lichen fairly abundant in the Venezuelan Andes above 2000 m. The availability of substantial amounts of this lichen facilitated the isolation and structural elucidation of consalazinic acid.

RESULTS AND DISCUSSION

Connorstictic acid

Although earlier work [2] resulted in the isolation of norstictic acid and an unidentified pigment, concretin, from *P. pseudocorallina*, TLC shows the presence of norstictic acid along with a more polar nonpigmented compound. Co-chromatography under standard conditions [3] showed this metabolite to be identical with the minor compound known as connorstictic acid found in *Parmelia perforata* (Jacq.) Ach. Continuous extraction of *P. pseudocorallina* with Me_2CO afforded a mixture of **1** and **2** which was recrystallized from Me_2CO–H_2O to give pure **2** identical (IR, NMR) with an authentic sample. Connorstictic acid was isolated from the mother liquor by repeated crystallisation and preparative TLC.

Elemental and spectral analysis were in agreement with a molecular formula of $C_{18}H_{14}O_9$. The similarity between the spectra of **1** and those of **2** indicated a close relationship between the two compounds. The IR showed depsidone ($1710\,cm^{-1}$) and lactone ($1745\,cm^{-1}$) carbonyls but lacked the strong absorption *ca* $1650\,cm^{-1}$ typical of the aldehyde of the β-orcinol depsidones. The ^1H NMR spectrum exhibited signals due to two aromatic Me groups at δ 2.20 and 2.37, the latter being weakly coupled to an aromatic proton at 6.73. A broad singlet at 7.05, sharpened on addition of D_2O and shifted to 7.79 in the tetra-acetate, is typical of the lactol proton of the β-orcinol depsidones. The remaining two-proton signal at 4.78, shifted to 5.30 in the tetra-acetate, is characteristic of an Ar–CH_2–OH function. Thus this spectrum was very similar to that of **1** the essential difference being the absence of the aldehyde group and its replacement by the benzyl alcohol function. Comparison of the ^{13}C NMR spectra of **1** and **2**, in both of which all 18 carbons were clearly distinguishable, also showed the replacement of the aldehyde at δ 192.8 in **1** by the benzyl carbon at 52.15 in **2**. These results are consistent with structure **2** for connorstictic acid. Catalytic reduction of norstictic acid in the presence of PtO_2 afforded a product identical in every way with connorstictic acid thus confirming the structure proposed for this compound.

Consalazinic acid

Continuous extraction of *P. subisidiosum* with toluene yielded atranorin identical with an authentic sample. Subsequent extraction of the lichen with Me_2CO afforded a brown solid consisting (TLC) of two compounds. The major component, salazinic acid (**3**), was isolated by recrystallization of the mixture from Me_2CO leaving the mother liquor considerably enriched in consalazinic acid (**4**). Preparative TLC and recrystallization from Me_2CO–H_2O gave pure **4**.

Consalazinic acid had a molecular formula of $C_{18}H_{14}O_{10}$ as indicated by combustion and spectral analysis. The IR showed depsidone and lactone carbonyls at 1692 and $1750\,cm^{-1}$, respectively, and as in the case of connorstictic acid the spectrum lacked any absorption attributable to an aldehyde group. Treatment of **4** with Ac_2O and H_2SO_4 as catalyst afforded a penta-acetate indicative of five hydroxyl groups. The ^1H NMR spectrum showed an aromatic proton at δ 6.65 coupled with an ArMe group at 2.35, this low field Me being *ortho* to the depsidone carbonyl. A broad four-proton multiplet centered at 4.66 was assigned to two Ar-$\underline{CH}_2$-OH moieties. Addition of D_2O resolved this multiplet into two broad overlapping peaks at 4.70 and 4.63 with the high-field signal considerably sharper than the other. The spectrum was completed by the lactol proton at 6.93 displaced to 7.67 in the penta-acetate. These results are compatible with structure **4** for consalazinic acid in which the aldehyde group of salazinic acid is replaced by a benzyl alcohol function. Confirmation of the proposed structure was obtained by reduction of the aldehyde group of salazinic acid with PtO_2 affording a compound identical (IR, NMR) with the natural product.

An interesting feature of the NMR spectra of both connorstictic tetra-acetate (**5**) and consalazinic penta-acetate (**6**) was the nonequivalence of the geminal methylene protons. The spectrum of **5** showed this group as an AB quartet (δ 4.82, 5.02, 5.58, 5.78; J = 12 Hz) while in **6** the presence of two such groups results in the appearance of two overlapping AB quartets (δ 5.78, 5.57, 4.97, 4.75; J = 12 Hz: 5.58, 5.35, 5.17, 4.95; J = 12 Hz). Similar patterns are found in the hexa-acetates of salazinic and penta- and tri-acetates of constictic acid although this same group in protocetraric acid penta-acetate appears as a broad singlet [4].

Recently we reported [5] the occurrence of hyposalazinic acid in *Xanthoparmelia quintaria* and speculated on its role in the biosynthesis of the β-orcinol depsidones. It appears reasonable to assume that conorstictic acid is derived from this compound by oxidation of 3-Me to an alcohol group and is in turn an immediate precursor of both norstictic acid and consalazinic acid, the latter affording salazinic acid on further oxidation.

Connorstictic and consalazinic acids are the first known β-orcinol depsidones substituted with a $-CH_2OH$ group at the methyl 3-α-3-position although Culberson [6] has recently reported the isolation of methyl-3-α-hydroxy-4-*O*-demethylbarbatate, a β-orcinol depside, from *Oropogon loxensis* (Fée) Th.Fr. Also, the related depside 3-α-hydroxybarbatic acid occurs in species of *Xanthoparmelia* [7].

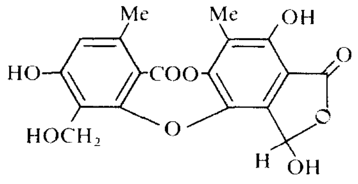
EXPERIMENTAL

Thin-layer chromatography. Although chromatography on Si gel HF_{254} with toluene–dioxane–HOAc (180:45:4) separates the β-orcinol depsidones it suffers from the disadvantage that salazinic (R_f = 0.11), connorstictic (R_f = 0.12), constictic (R_f = 0.06) and consalazinic acids (R_f = 0.02) are all grouped together at the bottom of the plate. We have found the system Si gel HF_{254}, toluene–EtOAc–HOAc (6:4:1) gives a better separation of these compounds: salazinic (R_f = 0.43), connorstictic (R_f = 0.40), constictic (R_f = 0.23) consalazinic (R_f = 0.13), norstictic (R_f = 0.56). After spraying with 10% H_2SO_4 and heating at 100° for 5 min connorstictic and consalazonic appear as intense red spots.

Isolation of connorstictic acid. The sterile crustose lichen *Pertusaria pseudocorallina* was collected on maritime rocks at Roberts Cove, Co. Cork, Ireland. TLC analysis of the lichen (Si gel HF_{254}; toluene–dioxane–HOAc; 180:45:5) showed norstictic acid along with a more polar compound (R_f = 0.1) appearing as a reddish spot after H_2SO_4 and heating at 100°. The previously reported [2] concretin was not detected either in the micro extract or during the subsequent large scale extraction. Powdered air-dried lichen (500 g) was continuously extract with Me_2CO in a Soxhlet apparatus and the extract concd under red, pres. On standing the solution deposited white crystals of norstictic acid, mp 280° d. (80% aq. Me_2CO). ^1H NMR (DMSO-d_6): δ 2.20 (3H, *s*, ArMe), 2.43 (3H, *s*, ArMe) 6.70 (1H, *br. s*, lactol H), 6.77 (1H, *s*, ArH), 10.9 (1H, *s*, CHO). ^{13}C NMR (DMSO-d_6): δ 9.62 (ring B Me), 21.44 (ring A Me), 95.25 (O—C—O), 109.16, 110.59, 111.76, 117.34, 120.98, 135.80, 137.36, 147.88, 152.03, 152.43, 160.36, 163.60, 164.12, 166.20, 192.84.

Norstictic penta-acetate. Prepared by reaction of **2** with Ac_2O–H_2SO_4, mp 212° (EtOH–H_2O). ^1H NMR ($CDCl_3$): δ 2.11, 2.15, 2.23, 2.33, 2.39, 2.47 (3H each, all *s*, 5 × AcO), 2.23 (3H, *s*, ArMe) 2.57 (3H, *s*, ArMe), 7.12 (1H, *s*, ArH), 8.0 (1H, *s*, lactol H) 8.12 (1 H, *s*, $(AcO)_2CH$). Evaporation of the mother liquor gave a solid containing approx. equal amounts of **1** and **2**. Prep. TLC (above solvent system) and crystallization from aq. DMSO afforded pure connorstictic acid, mp 280 300° d. (Found: C, 55.71; H, 3.70. Calc. for $C_{18}H_{14}O_9$: C, 57.75; H, 3.74%). IR ν_{max}^{KBr} cm^{-1}: 3400, 1745, 1710, 1610, 1445, 1250, 1292. ^1H NMR (DMSO-d_6): δ 2.20 (3H, *s*, ring B Me), 2.37 (3H, *s*, ring A Me), 4.78 (2H, *br. s*, Ar—CH_2—OH), 6.73 (1H, *s*, ArH), 7.05 (1H, *br. s*, lactol H). ^{13}C NMR (DMSO-d_6): δ 9.48 (ring A Me), 20.66 (ring B Me), 52.15 (—CH_2—OH), 98.77 (O—C—O), 108.99, 111.10, 115.65, 117.21, 120.33, 135.79, 138.13, 143.45, 148.53, 151.52, 159.83, 161.52, 166.72 (—CO_2Ar).

Isolation of consalazinic acid. The foliose lichen *P. subisidiosum* was collected on tree trunks in La Carbonera (alt. 2000 m), State of Mérida, Venezuela. TLC in the above solvent system showed the presence of atranorin in the cortex, and salazinic (**3**) along with consalazinic acid (**4**) in the medulla. Continuous extraction



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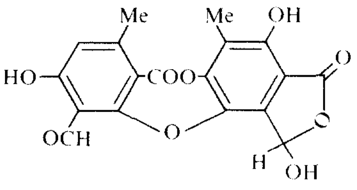

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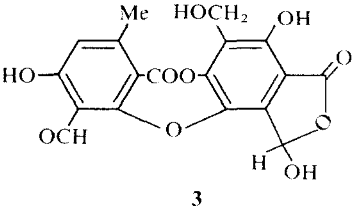

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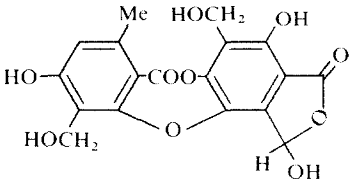

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with toluene removed the atranorin while subsequent extraction with Me_2CO afforded a mixture of **3** and **4**. Concentration of the extract deposited salazinic acid, mp 260–280°. ^1H NMR ($DMSO$-d_6): δ 2.45 (3H, *s*, ArMe) 4.70 (2H, *s*, Ar—CH_2—OH), 6.80 (1H, *s*, ArH), 6.83 (1H, *s*, lactol H), 10.83 (aldehyde). Hexa-acetate prepared with Ac_2O–H_2SO_4, mp 178°. ^1H NMR ($CDCl_3$): δ 1.98, 2.10, 2.12, 2.23, 2.38, 2.45 (3H each, all *s*, 6 × Me), 2.58 (3H, *s*, ArMe), 5.09 (1H, *d*, J = 12 Hz), 5.44 (1H, *d*, J = 12 Hz), 6.97 (1H, *s*, ArH), 7.87 (1H, *s*, lactol) 8.00 (1H, *s*). Triacetate prepared by refluxing **3** in Ac_2O, mp 205°. ^1H NMR ($CDCl_3$): δ 1.95, 2.11, 2.43, (3H each, all *s*, Ac), 2.59 (3H, *s*, ArMe), 5.27 (2H, *s*, Ar$\underline{CH}_2$—OH), 6.80 (1H, *s*, ArH), 7.49 (1H, *s*, lactol H). Evaporation of the mother liquor afforded a mixture of **3** and **4**. Prep. TLC (Si gel HF_{254}; toluene–EtOAc–HOAc; 6:4:1) followed by recrystallization from Me_2CO–H_2O afforded pure consalazinic acid, mp 260° d. (Found: C, 55.42; H, 3.61. Calc. for $C_{18}H_{14}O_9$: C, 55.38; H, 3.58%). ^1H NMR ($DMSO$-d_6): δ 2.35 (3H, *s*, ArMe), 4.63 (2H, *s*, Ar—CH_2—O), 4.70 (2H, *m*, Ar—CH_2—O) 6.65 (1H, *s*, ArH), 6.93 (1H, *br. s*, lactol H). IR ν_{max}^{KBr} cm^{-1}: 1750, 1692, 1610, 1475, 1260, 1130.

Consalazinic penta-acetate. The acid (300 mg) was suspended in Ac_2O (3 ml) containing one drop of H_2SO_4. Work-up in the normal manner gave the penta-acetate, white needles, mp 164°. (EtOH–H_2O) IR ν_{max}^{KBr} cm^{-1}: 1775 br, 1745 br, 1610, 1570, 1220, 1185: ^1H NMR ($CDCl_3$): δ 1.95, 2.07, 2.20, 2.30, 2.40, 2.57 (3H each, all *s*, 6 × Me) 4.75, 4.97, 5.57, 5.78 (2H, AB *quartet*, J = 12 Hz, Ar—$\underline{CH}_2$—OAc) 4.95, 5.17, 5.35, 5.58 (2H, AB *quartet*, J = 12.5 Hz, Ar—$\underline{CH}_2$—OAc) 6.97 (1H, *s*, ArH), 7.67 (1H, *s*, lactol H).

Connorstictic tetra-acetate. Connorstictic acid (100 mg) was suspended in Ac_2O (1 ml) and treated with 1 drop of H_2SO_4. On standing overnight the soln was worked up in the normal manner to afford the tetra-acetate, mp 238–240° (toluene–petrol). ^1H NMR ($CDCl_3$): δ 2.11, 2.25, 2.47 (3H each, all *s*, 3 × Me), 2.35 (6H, *s*, 2 × Me) 4.82, 5.02, 5.58, 5.78 (2H, AB *quartet*, J = 12.5 Hz, Ar—$\underline{CH}_2$—OAc) 7.12 (1H, *s*, ArH) 7.79 (1H, *s*, lactol H).

Synthesis of connorstictic and consalazinic acids. The corresponding aldehydes, norstictic and connorstictic acids, respectively, were suspended in EtOH (100 mg, 100 ml) and reduced in a Parr hydrogenator at 14 lb and room temp. during 6 hr in the presence of PtO_2 (Merck). The resulting clear soln was filtered and evapd to dryness affording after crystallization a compound identical in every way with the natural product.

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