PARTIE EXPERIMENTALE

Isolement de la L-γ-glutamyl-2-amino-3-hexanone. 2.6 kg de *R. ochroleuca* frais ont été broyés et extraits par de l'alcool à 95%. L'extrait filtré a été purifié sur une colonne de Lewatit S 1080, H^+ et les acides aminés ont été extraits par la pyridine 1 N. Les acides aminés aromatiques et le peptide ont été adsorbés sur une colonne de charbon traité par la méthode de Partridge. Après désorption, le mélange (0.5 g) a été fixé sur une colonne de Lewatit M 5080, forme AcO^-, 100–200 mesh, 4.5 × 95 cm. Par élution avic HOAc 0.5 N, le peptide passe avant les acides aminés aromatiques. (118 mg). C, 54.1; H, 8.37; N, 11.42. Calc. pour $C_{11}H_{20}O_4N_2$: C, 54.08; H, 8.25; N, 11.47%) IR: ν_{max}^{KBr} 3290 (*w*), 3270 (*m*), 3065 (*m*), 2960 (*m*), 1718 (*s*), 1640 (*s*), 1585 (*s*), 1535 (*m*), 1450 (*m*), 1408 (*s*), 1250 (*m*), 1122 (*m*), 1030 (*m*) cm^{-1}.

Méthodes d'analyses. La chromatographie sur papier Whatman 3 MM a été réalisée en utilisant comme solvant le mélange *n*-BuOH–HCOOH–H_2O (15:3:2) et le phénol saturé par un tampon à pH 4.2. Les R_f pour le dérivé, l'α aminocétone et l'acide glutamique sont respectivement: BuOH, 0.52; 0.63; 0.23; Phénol, 0.91; 0.77; 0.26.

Remerciement—Nous tenons à remercier le Professeur P. Heinemann, Faculté des Sciences Agronomiques de Gembloux et Mr. Marchal, Couvin pour la récolte et la détermination du champignon. Les spectres RMN ont été pris au service de Monsieur le Professeur Krief, Faculté Notre Dame de la Paix à Namur. Nous l'en remercions très sincèrement.

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THE STRUCTURE OF PARISHIN, A GLUCOSIDE FROM *VANDA PARISHII**

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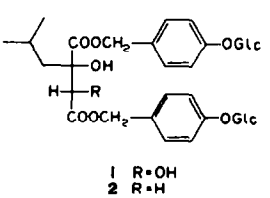
Key Word Index—*Vanda parishii*; Orchidaceae, glucoside, parishin.

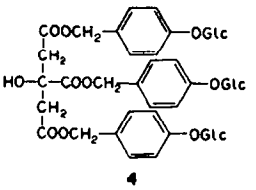
Abstract—Two new glucosides, tris [4-(β-D-glucopyranosyloxy)benzyl] citrate, named parishin, and 4-(β-D-glucopyranosyloxy)benzyl alcohol have been isolated from *Vanda parishii*. The latter compound may, however, be an artefact formed from parishin.

In a recent communication [2] Aasen *et al.* reported that the glucosides loroglossine (**1**) and militarine (**2**), both of which are found in *Orchis militaris* L., are diesters of 4-(β-D-glucopyranosyloxy)benzyl alcohol (**3**) and (2R, 3S)-2-isobutyltartaric acid and (*R*)-2-isobutylmalic acid respectively. We now report the occurrence in *Vanda parishii* of a new glucoside, named parishin (**4**), which is shown to be the triester of citric acid and **3**. Besides parishin (**4**), the glucoside **3** was isolated from the methanolic extract, but this substance may be an artefact formed from **4** during the isolation procedure.

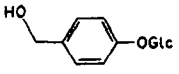
The glucosides **3** and **4** were isolated from a methanolic extract by chromatography on silica gel followed by



1 R=OH
2 R=H



4



3

* Part 7 in the series "Studies on Orchidaceae Glucosides". For part 6 see ref. [1].

gel permeation on Sephadex LH-20. Sugar [3,4] and methylation [5] analyses showed **3** and **4** to be glucopyranosides. The structure of **3** is evident from its elemental composition, spectral properties and the fact that it gave *p*-cresyl-β-D-glucopyranoside upon catalytic hydrogenation. Parishin (**4**) is a neutral substance which on catalytic hydrogenation followed by treatment with diazomethane yielded *p*-cresyl-β-D-glucopyranoside and trimethyl citrate in the molar ratio 3:1. The fission must be due to hydrogenolysis of benzyl esters, as no signals for aromatic methyl groups were observed in the NMR spectrum of **4**. These results demonstrate that **4** is tris[4-(β-D-glucopyranosyloxy)benzyl] citrate, a structure also consistent with the NMR spectrum.

EXPERIMENTAL

General conditions were the same as in an earlier communication [2].

Plant material. Vanda parishii. Rchb.f. was delivered from Mr N. Prakash, Chandra Orchid and Bulb Nurseries, 8 1/2 miles P.O. Kalimpong, West Bengal, India.

Isolation of **3** *and* **4**. Fresh plants of *V. parishii* (3 kg) were extracted with MeOH (10 l), and the solution was concentrated to 0.65 l. A part (100 ml) of this extract was diluted to 300 ml with water and washed with $CHCl_3$ (4 × 50 ml). The aqueous layer was saturated with butanol and extracted with butanol saturated with water (7 × 50 ml). The butanolic phase was washed with water (25 ml) and evaporated to dryness. A part (2 g) of the residue (6.4 g) was chromatographed on a silica gel column (5 × 8.5 cm) using $CHCl_3$–MeOH–H_2O (13:7:2, lower phase) as eluent. The fraction containing **3** (300 mg) was filtered through a column of Sephadex LH-20 (5 × 70 cm) using EtOH–H_2O (1:1) as eluent giving crude **3** (96 mg), which was crystallised from *iso*-PrOH–H_2O. Recrystallisation from EtOAc–EtOH gave **3** (56 mg). A part (220 mg) of the fraction containing **4** (450 mg) was chromatographed on silica gel (2.6 × 11 cm) using the same eluent as above. The fraction containing **4** (150 mg) was filtered through a column of Sephadex LH-20 (2.5 × 83 cm) using EtOH–H_2O (1:1) as eluent, giving **4** as a colourless amorphous solid (78 mg).

Glucoside **3**. Needles (EtOAc–EtOH), mp 154–157°C; $[\alpha]_{578}^{22}$ − 63° (*c* 0.77, MeOH). (Found: C 54.6; H 6.3; O 39.1. $C_{13}H_{18}O_7$ requires: C 54.5; H 6.3; O 39.1). IR: ν_{max}^{KBr} 3700–3000(*s*), 1615(*m*), 1590(*m*), 1510(s) cm^{-1}. UV: λ_{max}^{MeOH} (log ε) 277.5 (2.96), 271 (3.04) nm. ^1H NMR (D_2O): δ 3.36–4.12 7.19 and 7.42 (4 H, A_2B_2 system, *J* 9 Hz).

Parishin (**4**). Amorphous solid, $[\alpha]_{578}^{22}$ −59° (*c* 0.80, MeOH). IR: ν_{max}^{KBr} 3700–3000(*s*), 1735(*s*), 1615(*m*), 1590(*m*), 1515(*s*) cm^{-1}. ^1H NMR (D_2O): δ 2.76 and 2.94 (4 H, two AB systems, *J* 15 Hz), 3.4–4.0 (18 H), 4.4–5.1 (the benzylic and the anomeric protons; the HOD signal partially overlapping), 6.9–7.4 (12 H). ^1H NMR (Pyridine-d_5): δ 3.32 and 3.37 (4 H, two AB systems, *J* 15 Hz), 3.80–4.66 (18 H), 5.09 (*s*, 4 H), 5.28 (*s*, 2 H), 5.58 (*d*, 3 H, *J* 6 Hz), 7.1–7.5 (12 H).

Hydrogenation of **3**. A soln of **3** (51 mg) in MeOH (9 ml) was hydrogenated over Pd (20 mg, 10% on carbon) at room temp. and atm. pres. After 7 hr the catalyst was filtered off and the soln was evaporated to dryness giving *p*-cresyl-β-D-glucopyranoside, mp 180–182°C (*iso*-PrOH–H_2O); $[\alpha]_D^{22}$ −67° (*c* 0.76, H_2O) (lit. [6] mp 178–179.5°C; $[\alpha]_D^{20}$ − 67.7° (H_2O)), further identified by NMR.

Hydrogenation of **4**. Parishin (92 mg) was hydrogenated as described for **3**. Catalyst was filtered off and the soln was treated with an excess of CH_2N_2 in Et_2O and evaporated to dryness. Residue was washed with $CHCl_3$ (5 × 1 ml) and the $CHCl_3$ phase was evaporated to dryness. Residue was crystallised from Et_2O–hexane at −20°C giving trimethyl citrate (14 mg), mp 76–78°C (lit. [7] mp 78.5–79°C). The total amount of trimethyl citrate was found by GLC to be 17.5 mg. The residue insoluble in $CHCl_3$ above was dissolved in H_2O and the soln washed with $CHCl_3$–MeOH (1:1). The aq phase was evaporated to dryness giving *p*-cresyl-β-D-glucopyranoside (68 mg), mp 181–182.5°C; $[\alpha]_D^{22}$ − 66° (*c* 0.27, H_2O).

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NEOCAPILLEN, A NEW ACETYLENIC HYDROCARBON FROM *ARTEMISIA CAPILLARIS*

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Key Word Index—*Artemisia capillaris*; Compositae; acetylenic hydrocarbon; neocapillen.

Abstract—During an investigation of *Artemisia capillaris*, a new acetylenic hydrocarbon, neocapillen, was isolated as a minor component and its structure determined.