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A NOVEL PHENOLIC ACID DERIVATIVE FROM BUDS OF *POPULUS LASIOCARPA*

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Key Word Index—*Populus lasiocarpa*; Salicaceae; bud excretion; novel phenolic; 1,3-*p*-coumaryl-2-acetyl-glycerol.

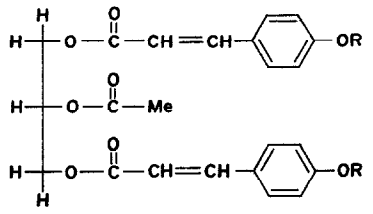
Abstract—The lipophilic excretion of winter buds of *Populus lasiocarpa* in contrast to most other species of the genus does not contain flavonoid aglycones. One of the compounds which are excreted in this plant now could be isolated and identified by chemical methods (hydrolysis, hydrogenation) and spectral analysis (UV, IR, NMR, MS) to be 1,3-*p*-coumaryl-2-acetyl-glycerol.

INTRODUCTION

In previous papers the isolation of about 30 flavonoid aglycones from buds of poplars has been reported. These investigations have led to a comprehensive chemotaxonomic survey of many species and clones [1]. It has been mentioned there that in some members of certain sections (especially Leuce and Leucoides) the bud excretion contains phenolic acids and their derivatives rather than flavonoids. Now we isolated and identified a 1,3-diacyl-2-monacetin **1** from *Populus lasiocarpa* Oliv., a species native in N and W China.

RESULTS AND DISCUSSION

Extraction and chromatographic separation yielded one of the substances excreted by this species as colourless crystals, mp 158–159°. The molecular formula was $C_{23}H_{22}O_8$ (high resolution MS and elemental analysis). The IR spectrum showed an acetyl group (1730 cm^{-1}), a conjugated ester (1715 cm^{-1}), a double bond (1640 cm^{-1}), a para-substituted benzene ring (835 cm^{-1}) and a hydroxy group (3400 cm^{-1}). The UV λ_{max} at 318 nm and the base peak in the MS at $m/e = 147$ ($OH{-}C_6H_4{-}CH{=}CH{-}CO^+$) indicated the presence of a *p*-coumaryl-moiety. The signals in the NMR which indicated two coumarate moieties were a singlet at δ 8.89 (2 H), due to two phenolic hydroxy protons, AB doublet at 7.66 (2 H, *J* 16 *Hz*) *and* 6.38 (2 *H*, *J* 16 Hz), assigned to two *trans*-ethylenic protons adjacent to carbonyl group and benzene ring, AB doublet at 6.91 (4 H, *J* 8 Hz) and 7.54 (4 H, *J* 8 Hz), assigned to each four protons on the two *p*-substituted benzene rings. The NMR spectrum also showed the presence of an acetyl group (3 H, *s*, 2.07), two methylene groups (2 H, *d,d*, 4.26, *J* 13 and 5 Hz) (2 H, *d,d*, 4.52, *J* 13 and 4 Hz) and one methine group (*m*, 5.38), which were adjacent to ester oxygen. The signal pattern of the methylene and methine protons was very similar to those of triacetin and tristearin. All the assignments were accomplished by the double resonance experiment. The presence of two phenolic hydroxy groups and ethylenic double bonds was also confirmed by acetylation and by catalytic hydrogenation to give the triacetate **2**, and the tetrahydro derivative respectively. Hydrolysis of **1** with alkali gave *p*-coumaric acid, glycerol and acetic acid. The location of the acetyl group at C-2 was supported by the absence of optical rotation [2] and optical rotatory dispersion [3–5] and by the complete identification of the fine structure of NMR signals of methine proton of the tetrahydro derivative with that of triacetin sweeped at 250 MHz.



(**1**) R = H
(**2**) R = Ac

Diacyl monoacetins have been rarely found in plants or animals. It is known that Celastraceae, Lardizabalaceae, Ranunculaceae and Rosaceae plants contain monoaceto triglycerides [3]. Recently liliosides, 2- or 3-acetyl glycerides, were isolated from *Lilium longiflorum* [4]. Optical active (*S*)-1,2-diacyl-3-acetins, distearoacetin, stearo-oleaoacetin and stearo-linaleo-acetin have been isolated from lipids of the insect *Icerya purchasi* [5]. It is interesting from biochemical and physiological viewpoints that buds of *Populus lasiocarpa* excrete remarkable amounts of the 1,3-di-*p*-coumaryl-monoacetin described here. Previously free *p*-coumaric acid as well as ferulic acid, isoferulic acid, caffeic acid and chlorogenic acid have been found in buds of several species of *Populus* [6] and other phenolics have been known before from buds of *P. balsamifera* [7]. Other components of *P. lasiocarpa* are now under investigation.

EXPERIMENTAL

Buds of *Populus lasiocarpa* Oliv., grown at the Botanical Garden of the University of Heidelberg, were collected in March and extracted with Me_2CO. The conc extract was

chromatographed over Si gel with C_6H_6 and increasing quantities of MeCOEt and MeOH. Some of the fractions, after long standing, showed a tendency to crystallize, but hitherto only one compound could be obtained in a pure state, after repeated crystallization from dil HOAc. This is the monoacetin **1** described here.

Compound **1**. Mp 158–159° $[\alpha]_D = 0.0$ ($c = 0.26$ *in* MeOH). High resolution MS gave MW 426.1347, calculated for $C_{23}H_{22}O_8$: 426.1314. UV λ_{max}: 231 nm (log ϵ 4.27) and 318 nm (log ϵ 4.55). MS 70 eV *m/e* (rel. int.): 426 M^+ (4), 366 ($C_{21}H_{18}O_6$, M–Me–COOH; 1), 263 ($C_{14}H_{15}O_5$, M–OH–C_6H_4–CH=CH–CO–O; 3), 164 ($C_9H_8O_3$, OH–C_6H_4–CH=CH–COOH; 14), 147 ($C_9H_7O_2$, OH–C_6H_4–CH=CH–CO^+; base peak), 119 (C_8H_7O, OH–C_6H_4–CH=CH; 23), 91 (20), 65 (10), 43 (34). Elemental analysis: C 64.74%, H 5.18%; calculated for $C_{23}H_{22}O_8$: C 64.78%, H 5.20%.

Acetylation of **1** with Ac_2O–C_6H_5N at room temp. for 48 hr gave, after purification by preparative TLC and after recrystallization from hexane–Et_2O colourless needles of the diacetate **2**. Mp 91–92°, $[\alpha]_D = 0.0$ ($c = 0.80$). UV λ_{max} 219 nm (log ε 4.74), 225 nm (log ε 4.83), 285 nm (log ε 4.99). IR ν_{max}: 1770 (AcO–C_6H_4–), 1745 (CH–OAc), 1720 (OOC–CH=CH–C_6H_4–), 1640, 1610, 1501, 1165, 835. NMR (90 MHz, $CDCl_3$, TMS): δ 2.08 (3 H, *s*, CH–OAc), 2.26 (6 H, *s*, 2 × AcO–C_6H_4–), 4.32 (2 H, *d,d*, *J* 11 & 6 Hz), 4.46 (2 H, *d,d*, *J* 12 Hz and 4 Hz), 5.37 (H, *m*), 6.36 (2 H, *d*, *J* 16 Hz), 7.08 (4 H, *d*, *J* 9 Hz), 7.50 (4 H, *d*, *J* 9 Hz), 7.65 (2 H, *d*, *J* 16 Hz). MS 70 eV *m/e* (rel. int.): 510 M^+ (1), 468 (monoacetate, 10), 305 (fragment from monoacetate, corresponding to 263 in **1**, 1), 189 (corresponding to 147 in **1**, 18), 147 (base peak), 119 (23), 91 (20), 65 (10), 43 (34). Elemental analysis: C 63.48%, H 5.16%; calculated for $C_{27}H_{26}O_{10}$: C 63.52%, H 5.13%. Hydrogenation in MeOH in the presence of 10% Pd–C at room temp. was carried out until 2 mol H_2 were absorbed. The product was purified by prep. TLC using C_6H_6–EtOAc–hexane 14:7:6 as solvent to give the tetrahydro derivative as a colourless oil. $[\alpha]_D = 0.0$ ($c = 0.45$). UV λ_{max}: 226 nm (log ϵ 4.11), 279 nm (log ϵ 3.45), 286 nm (log ϵ 3.36) [cf. *p*-hydroxytoluene 279 nm (log ϵ 3.29), 286 nm (log ϵ 3.25)]. IR ν_{max}: 1740 (acetate), 1710 (coumarate), 1630, 1595, 1585, 1180, 825 cm^{-1}. NMR (90 MHz, $MeCOCD_3$, TMS): δ 1.96 (3 H, *s*), 2.6 and 2.79 (A_2B_2, 8 H), 4.08 (2, *d,d*, *J* 12 & 6 Hz), 4.26 (2 H, *d,d*, *J* 12 & 4 *Hz*), 5.15 (H, *m*), 6.71 (4 H, *d*, *J* 9 Hz), 7.20 (4 H, *d*, *J* § Hz). MS 70 eV *m/e*: 430 M^+, 149, 121 (base peak), 117, 107, 91, 43. Analysis: C 64.32%, H 6.14%; calculated for $C_{23}H_{26}O_8$: C 64.17%, H 6.09%. Alkaline hydrolysis of **1** was with 5% methanolic KOH under reflux for 8 hr. After acidification and extraction with Et_2O a small amount of acetic acid (identified by GLC) and *p*-coumaric acid (identified by IR, UV and mp) were obtained. The mother liquor yielded a viscous liquid whose IR was identical to that of glycerol.

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