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SHORT REPORTS

FREE PYRIMIDINE NUCLEOSIDE AND NUCLEOTIDES FROM *CICER ARIETINUM*

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Key Word Index—*Cicer arietinum*; Leguminosae; 5-β-D-ribofuranosyluracil (pseudouridine); uracil 5-β-D-fructofuranosyl-1′-monophosphate and uracil 5-β-D-ribofuranosyl-2′,3′ cyclic-monophosphate.

Abstract—5-β-D-Ribofuranosyluracil (pseudouridine) and the new nucleotides uracil 5-β-D-fructofuranosyl-1′-monophosphate and uracil 5-β-D-ribofuranosyl-2′,3′-cyclic monophosphate have been isolated in the free state from *Cicer arietinum* seeds and characterized by spectroscopic methods.

INTRODUCTION

Al-Baldawi and Brown [1] in a recent communication have for the first time reported the accumulation of free pseudouridine (5-β-D-ribofuranosyl-uracil) in *Phaseolus vulgaris* although pseudouridine has been reported as a constituent of the hydrolysates of RNA. In continuation of our investigations on the nucleotides [2] of *Cicer arietinum* L., we have isolated from this source two free pyrimidine nucleotides, uracil 5-β-D-fructo-furanosyl-1′-monophosphate and uracil 5-β-D-ribofuranosyl-2′,3′-cyclic monophosphate, not reported before in plants, as well as the free nucleoside, pseudouridine.

RESULTS AND DISCUSSION

Compound **1**† was identified as uracil 5-β-D-fructofuranosyl-1′-monophosphate, mp 308–310 (d). The presence of a chromophoric group identical to uracil was indicated by the following spectroscopic data: UV $\lambda_{max}^{H_2O}$, pH 7.0, 262 nm; IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 2800–2560 (P–OH), 1680, 1630 (>C=O, NH) and 1280 (P=O); ^1H NMR (Jeol Fx 90 Q; C_5D_5N): δ2.6 (*s*, 2H, H-1′), 3.0–4.0 (*m*, 3H, H-3′, H-4′, H-5′) and 7.6 (*s*, 1H, H-6). The mass spectrum (EI 70 eV) of **1** contained no $[M]^+$ peak but the presence of peaks at *m/z* 110 $[CH_2OPO_3H]^+$ (2.4), 111 $[B]^+$ (0.5), 112 $[B+1]^+$ (0.8), 113 $[B+2]^+$ (0.5), 140 $[BCHO]^+$ (0.14), 141 $[BHCHO]^+$ (0.14) and 142 $[BH_2CHO]^+$ (0.40) confirmed the presence of uracil whilst those at *m/z* 45 (bp 100), 60 (8.36), 84 (10.67) 85 (6.53), 119 (0.64), 133 (0.34) and 179 (0.06) confirmed the presence of the sugar moiety.

The mass spectrum of the TMS derivative of **1** did not give any $[M]^+$ peak at *m/z* 714 (**1** + TMS 4)/786 (**1** + TMS 5). The presence of peaks at *m/z* 445 (0.3) and 437 (0.78) were a further indication of **1** being a 2-ketohexoside as reported by Krady and Pines [3] and Lawson *et al.* [4]. Other characteristic peaks appeared at *m/z* 140 $[BCHO]^+$ (67.2), 141 $[BHCHO]^+$ (over), 142 $[BH_2CHO]^+$ (27.75), 183 $[BTMS]^+$ (2.95), 184 $[BHTMS]^+$ (3.28), 185 $[BH_2TMS]^+$ (4.74), 213 $[BTMS - CHOH]^+$ (over), 214 $(BHTMS - CHOH]^+$ (43.5) for the uracil moiety and at *m/z* 73 (bp 100), 116 (71.7), 132 (12.3), 204 (43.5), 217 (2.88), 255 $[CH_2O(TMS)_2 - P=O]^+$ (12.33), 298 (11.16), 387 (3.27, tetrakis phosphate ion), 411 (0.018), 413 (0.006), 430 (0.02), 437 (0.78), 438 (0.30) 439 (0.16), 445 (0.30), 446 (0.12), 460 (0.16), 461 (0.004) and 532 (0.10) for the 2-ketohexoside.

In the high resolution mass spectrum **1** gave *m/z* values of 354.0483 ($C_{10}H_{15}N_2O_{10}P$, $[M]^+$), 279.9832 ($C_8H_{11}NO_8P$), 221.0019 ($C_5H_6N_2O_6P$) and 207.0106 ($C_6H_8O_6P$) which were in agreement for uracil 5-β-D-fructo furanosyl-1′-monophosphate.

Compound **2** was identified as pseudouridine, mp 220–222° (d): UV $\lambda_{max}^{H_2O}$ pH 7.0: 262 nm; **2** HCl, mp 158–160°, $[\alpha]_D^{25}$ −38° (H_2O); $\lambda_{max}^{H_2O}$ pH 7.0: 260 nm; IR ν_{max}^{KBr} cm^{-1}: 3650–3050 (OH) 1680, 1620 (>C=O, NH), 1600 and 1490 (=bond). The presence of ^1H NMR (60 MHz, C_5D_5N) signals at δ3.25–4.0 (*m*, H, H-1′, H-5′) and 7.48 (*s*, 1H, H-6) indicated a C-6/C-1, linkage as reported by Cohn [5] and Hruska *et al.* [6] for pseudouridine. The ^{13}C NMR (Jeol, FX 90 Q, D_2O) spectrum contained signals at δ156.0 (C-2′), 160.5 (C-4, >C=O), 107.5 (C-5, *s*), 127.5 (C-6, *d*), 87.0 (C-1′, *d*), 70.8 (C-2′, *d*), 75.5 (C-3′, *d*), 84.8 (C-4′, *d*), and 61.8 (C-5′, *t*).

Compound **2** gave no $[M]^+$ peak in the mass spectrum but the peaks for the uracil fragment appeared at *m/z* 40 (0.05), 41 (0.08) 42 (0.31), 68 (0.01), 69 (0.05) and 111 (0.01) and for the sugar at *m/z* 36 (bp 100°), 60 (0.06), 73 (0.05), 91 (0.01), 119 (0.01), 128 (0.03), 129 (0.01) & 151 (0.29).

The TMS derivative of **2** gave no $[M]^+$ peak at *m/z* 460 (**2** + TMS_3)/532 (**2** + $TMS)_4$ but characteristic peaks appeared at *m/z* 60 (13.8), 68 (33.3), 73 (over), 75 (84.48), 98 (24.29), 130 (2.69), 131 (43.0), 132 (27.12), 133 (5.1), 140 $[BCHO]^+$ (71.35), 141 $[BHCHO]^+$ (over), 142

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†Compounds **1**–**3** are well known and are not illustrated.

$[BHCHO]^+$ (21.37), 143 $[BH_2CHO]^+$ (18.57), 147 (0.16), 183 (6.31), 184 (4.2), 185 (2.1), 189 (1.81), 199 (4.52), 204 (20.57), 213 $[BTMS]^+$ (53.2), 214 $[BHTMS]^+$ (11.92), 215 $[BH_2TMS]^+$ (14.2), 265 (6.92), 350 (S, 0.06).

The high resolution mass spectrum values of *m/z* 244.1023 ($C_9H_{12}N_2O_6$, $[M]^+$), 227.0838 ($C_9H_{11}N_2O_5$), 207.0090 ($C_7H_{11}O_8$) and 155.0014 ($C_6H_7N_2O_3$) agreed with the structure 5-β-D-ribofuranosyl-uracil (pseudouridine).

Compound **3** was identified as uracil 5-β-D-ribofuranosyl-2′,3′-cyclic monophosphate [7], mp 220–222° (d). UV $\lambda_{max}^{H_2O}$ pH 6.5–7.0: 262 nm: IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 1680 and 1620 (>C=O, NH); ^1H NMR (60 MHz D_2O): δ 7.6 (*s*, 1H, H-6). It gave no $[M]^+$ peak in the mass spectrum but the characteristic peaks appeared at *m/z* 28 (bp 100), 43 (70.32), 58 (23.0), 69 (1.64), 73 (17.0), 98 (0.2), 110 (B, 0.82), 133 (5.85) 142 $[BHCHO]^+$ (3.16), 147 (3.86), 151 (1.81) and 207 (42.43).

The TMS derivative for compound **3** gave no $[M]^+$ peak at *m/z* 450 (3 + TMS 2)/522 (3 + TMS 3) but the peaks appearing at *m/z* 507 $[M\,(3 - TMS\,3) - Me]^+$ (0.002), 433 $[M(TMS\,2) - (TMSiOH_2)]^+$ (0.06) and 361 $[M\,(TMS)_2 - TMSiOH_2]^+$ (0.06) were suggestive of the $[M]^+$ peak. The presence of peaks at *m/z* 318 (0.66), 321 (0.08) and 393 (0.1) were indicative of the 2′,3′ cyclic monophosphate moiety. Other characteristic peaks appeared at *m/z* 43 (27.0), 73 (over), 75 (75.2), 98 (43.5), 112 (1.34), 113 (1.71), 116 (73.5), 125 (34.4), 141 $[BCHO]^+$ (4.5), 183 $[BTMS]^+$ (4.5), 184 $[BHTMS]^+$ (1.3), 185 $[BH_2TMS]^+$ (2.7), 204 (19.0), 213 $[BH_2TMS - CHO]^+$ (79.59), 214 $[BHTMS - CHO]^+$ (17.89), 215 $[BH_2TMS - CHO]^+$ (19.8), 217 (2.54), 265 (15.7), 287 (9.78), 305 (0.9), 339 (s, 0.07), 343 (0.06), 344 (0.06), 347 (0.07), 348 (0.02), 431 (0.12), 432 (0.14) and 506 (0.09).

The high resolution mass spectrum values of *m/z* 245.0053 ($C_8H_8NO_6P$), 207.0106 ($C_5H_8N_2O_5P$) and 195.0086 ($C_5H_8O_6P$) provided further support for **3** being uracil 5-β-D-ribofuranosyl-2′,3′-cyclic monophosphate.

EXPERIMENTAL

C. arietinum (Bengal gram) seeds (10 kg) were soaked overnight in H_2O (20 l.) and extracted (× 3) with hot H_2O (30, 20, 20 l.) in an open steampan. The combined H_2O extract was cooled, strained through fine muslin cloth, concd to about 10 l. and adjusted to pH 4.0 with $HClO_4$ and centrifuged to give a clear supernatant liquid ('A').

Isolation of **1**. About half of liquid 'A' was passed over a column (100 × 10 cm) of activated charcoal decolourising powder (200 g, BDH, LR) and the column washed with H_2O. The material adsorbed over charcoal was eluted with EtOH–H_2O (1:1) containing NH_4OH (1%). The ammoniacal eluate was adjusted to pH 5.0 with AcOH and dried *in vacuo* to give a dark brown amorphous powder (80 mg), mp 308–310° (d); UV $\lambda_{max}^{H_2O}$ pH 7.0: 262 nm.

Isolation of **2**. The remaining half of liquid 'A' was treated with $CaCO_3$ to pH 5.6, centrifuged and the supernatant concentrated (~ 2 l.) and treated with EtOH (10 l.). The precipitate was removed by filtration, dried and dissolved in the minimum quantity of H_2O. The mixture was clarified by centrifugation and the supernatant passed over an Amberlite IR-120 (200 g, BDH, H^+ form) column (6.6 × 75 cm) to give eluate 'B'. The column was washed with H_2O and the washings added to 'B'. The material adsorbed on the column was eluted with aq. HCl (4%), the column washed with H_2O and the washings mixed with the aq. acid eluate. The aq. acid eluate was passed over an activated charcoal (18 g, BDH, LR)–celite (2 g, 545 filter aid, Loba) column (2.54 × 40 cm) and the eluate on evaporation of the liquid yielded a white crystalline compound from MeOH (**2**, 70 mg), mp 220–222°; UV $\lambda_{max}^{H_2O}$ pH 7.0: 262 nm; **2**-HCl (MeOH) mp 158–160° (d), $[\alpha]_D^{20}$ – 38.0 (H_2O): UV $\lambda_{max}^{H_2O}$: pH 6.5–7.0: 260 nm.

Isolation of **3**. The eluate 'B' was passed over an Amberlite IR-400 (200 g, BDH, OH-form, 6.6 × 75 cm) column and the column washed with H_2O. The material adsorbed on the column was eluted with 0.001 N, 0.01 N and 0.1 N HCl and the fractions pooled on the basis of UV absorbance at 260 nm.The pooled fractions were passed over an activated charcoal (18 g, BDH, LR)–celite (2 g, 545 filter acid, Loba) column (2.54 × 40 cm), and washed with H_2O. The material adsorbed on the column was eluted with EtOH–H_2O (1:1) containing NH_4OH (1%); adjusted to pH 5.0 with AcOH; dried *in vacuo* to give a white crystalline material from MeOH (50 mg), mp 220–222° (d). UV $\lambda_{max}^{H_2O}$ pH 7.0: 262 nm. The methanolic mother liquor further yielded **1** mp 308–310° (d); UV $\lambda_{max}^{H_2O}$ pH 6.5–7.0: 260 nm.

Preparation of TMS derivatives. Compounds **1**–**3** were treated with BSFTA (*N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethyl chlorosilane, Fluka) in sealed vials and left for about 1 hr with occasional shaking after which the samples were ready for mass spectroscopic studies.

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