4-Alkoxy-1,3-dimethyl-1,3-dihydro-2H-imidazo[4,5-c]pyridin-2-ones (VIIIb-e). To the solution obtained by heating 0.15 mole of NaOH (or KOH) in 1.8-2.0 mole of the appropriate alcohol was added 0.1 mole of Vd, and the mixture was boiled for 2-4 h. The sodium (or potassium) nitrate precipitate was filtered off, excess alcohol was distilled from the filtrate the reaction product was extracted with hot chloroform (50-60 ml) and the solvent was evaporated.

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SYNTHESIS OF N_6-SUBSTITUTED ADENINYL-9-β-D-GLUCOFURANURONOSIDES

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N_6-substituted adeninyl-9-β-D-glucofuranuronosides have been obtained by the condensation of trimethylsilylated 6-aminopurines with 1,2,5-tri-O-acetyl-β-D-glucofurano-6,3-lactone. The structure of the glucuronides was demonstrated by the UV, IR, and PMR spectra.

For the synthesis of nucleosidic derivatives of kinetine (6-furfurylaminopurine) and other 6-substituted adenines, mainly the amination of 6-chloro- and 6-methylmercaptopurine nucleosides has been used [1-4]; only in individual cases [5] has glycosylation of a 6-substituted adenine with a carbohydrate fragment been used.

The purpose of the present work was to synthesize potential cytokinines in the 6-substituted adenine glucuronide series. Attempts to use 1-(6-chloro- or 6-methylmercaptopurinyl-9)-β-D-glucofuranosides that we had previously synthesized [6] to obtain these compounds were unsuccessful, due to the instability of the glycoside bond and the lactone ring under the reaction conditions. We therefore synthesized the compounds by condensation of the trimethylsilyl derivatives of 6-methylamino- (IIa), 6-butylamino- (IIb), 6-cyclohexylamino- (IIc), 6-benzylamino- (IId), 6-morpholino- (IIe), and 6-furfurylaminopurine (IIf) with 1,2,5-tri-O-acetyl-β-D-glucofurano-6,3-lactone (III) [7] in 1,2-dichloroethane in the presence of the condensing agent trimethylsilyltrifluoromethanesulfonic acid (TMS-TF), which is more reactive than $SnCl_4$ which we used previously [6, 8].

When the reaction was carried out at 80° for 12 h (II:III:TMS-TF 1.1:1.0:1.2 moles), the principal product was the N_9-β-D-glucofuranoside of N_6-substituted purines (IVa-f). The TLC data bear witness that other nucleosidic products are formed in the reaction (not more than

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TABLE 1. Parameters of PMR Spectra of Compounds IVa-f

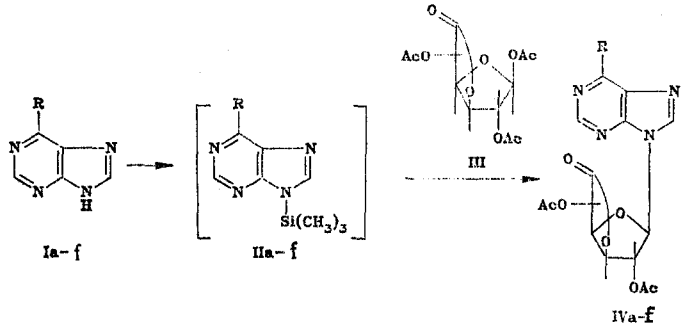
Compound	Chemical shift, δ, ppm							SSCC J, Hz			
	2-H s, 8-H s	1'-H d	2'-H	3'-H	4'-H dd	5'-H d	other protons	1'2'	2'3'	3'4'	4'5'
IV[a]	8,23, 8,15	6,34	6,13 dd	5,28 dd	5,15	5,89	2,12, s (3H, OAc), 2,09, s (3H, OAc), 2,97, m (3H, CH_3), 7,84, m (1H, NH)	4,0	~0,75	3,8	5,4
IVb	8,20, 8,16	6,31	6,11 dd	5,27 dd	5,13	5,88	2,11, s (3H, OAc), 2,06, s (3H, OAc), 0,88, t (3H, CH_3), 1,37—1,70 (4H, $2CH_2$), 3,48, m (2H, CH_2), 7,88, m (1H, NH)	4,0	1,0	3,4	4,8
IVc	8,21, 8,17	6,32	6,11 d	5,29 dd	5,15	5,91	2,12, s (3H, OAc), 2,09, s (3H, OAc), 1,17—1,95 (10H, $5CH_2$), 4,13, m (1H, CH), 7,67, d (1H, NH)	3,8	0,75	3,4	4,8
IVd	8,20, 8,18	6,32	6,11 d	5,26 d	5,12	5,86	2,10, s (3H, OAc), 2,07, s (3H, OAc), 4,74, m (2H, CH_2), 7,24, m (5H, Ph), 8,47, m (1H, NH)	4,0	~1,0	3,6	5,0
IVe	8,28, 8,22	6,37	6,11 dd	5,29 d	5,17	5,84	2,12, s (3H, OAc), 2,09, s (3H, OAc), 3,73, m (4H, $2CH_2$), 4,22, m (4H, $2CH_2$)	3,6	0,75	3,4	5,0
IVf	8,26, 8,20	6,33	6,13 dd	5,28 d	5,16	5,90	2,10, s (3H, OAc), 2,06, s (3H, OAc), 4,75, m (2H, CH_2), 6,26—6,38 (3H, 3CH), 7,54, t (1H, NH)	3,8	0,75	3,8	5,0

5-10% of the total); these could not be separated in pure form for identification. Apparently they are isomers of nucleosides IVa-f. Furthermore, unreacted lactone III and 6-aminopurines Ia-f are left in the reaction mixture. Changing the proportions of reagents or raising the reaction temperature to boiling did not increase the yield of nucleosides IVa-f. The glucuronide of 6-dimethylaminopurine could not be synthesized by this method, because the silyl derivative of 6-dimethylaminopurine does not react with lactone III even at the boiling point of the solvent. Analytically pure samples of IVa-f were obtained by column chromatography of the reaction products on silica gel.

The structure and configuration of the synthesized nucleosides were established on the basis of spectral data.

The properties of the UV absorption spectra of the 6-aminopurine nucleosides IVa-f are identical with those of the N_9-alkyl derivatives [9, 10] and the N_9-ribofuranosides [2-5, 11, 12] of the related heterocyclic bases, and are significantly different from those of the N_7 derivatives [5, 13].

The IR spectra of IVa-f contain absorption bands at 1595-1615 (purine C=N), and in the 1800-1805 (γ-lactone C=O) and 1750-1760 cm^{-1} (acetate C=O) regions.



I-III a R = $NHCH_3$; b r R = HNC_4H_9; c R = HNC_6H_{11} i d R = $HNCH_2C_6H_5$; i R = morphlino –; f R = furfurylamino –

The PMR spectral data (Table 1) confirm the β-anomeric configuration and the presence of a hexafuranose ring in these compounds [14]. The spin–spin coupling constant (SSCC) values of the carbohydrate protons of IVa-f (at least 6 Hz) are typical of furanose derivatives. This enables us to confirm that during the glycosylation of the purine bases the

furanose form is not converted to pyranose. The SSCC value $J_{1'2'}$ = 3.6-4.0 Hz is evidence for the β-anomeric configuration of the lactones IVa-f.

Cytokinine properties were studied for IVd,f by the tissue culture method in synthetic culture media, for the growth of plants from meristem. The tests were carried out on the growth of Dianthus from meristem in a modified culture medium. To compare the action of glucuronides IVd and f, kinetin was added to the medium. The tests show that at concentrations of 0.125 and 0.250 ml/g, IVd and IVf act approximately at the level of kinetine.

Another experiment was carried out to study lactones IVd,f as stimulators of sprout formation in the growth of cuttings from meristem. In this case 6-benzylaminopurine (BAP) was added to the culture medium to stimulate sprout formation. It was determined that the activities of IVd and IVf are lower than that of BAP.

EXPERIMENTAL

UV spectra were recorded in methanol on a Spectromom-204 spectrophotometer; IR spectra in mineral oil on a Perkin-Elmer spectrometer; PMR spectra in DMSO-D_6 on a Bruker WH-90 instrument, with HMDS internal standard. Specific rotation was determined on a Perkin-Elmer 241 spectropolarimeter.

The course of the reaction and the identity of the reaction products were monitored by TLC on Silufol-254 plates in a 9:1 chloroform-methanol system. The chromatograms were developed by spraying with a 1:1 mixture of 0.2% naphthoresocinol in ethanol and dilute (1:10) phosphoric acid followed by heating at 110-115° for 15 min. Column chromatography was carried out on a LKB (Switzerland) column (2.5 × 60 cm) with L100/250 silica gel (Czechoslovak SSR).

6-Aminopurines Ia-f were synthesized by the procedures of [15, 16] from 6-chloropurine or 6-methylmercaptopurine.

Silylation of 6-aminopurines Ia-f was carried out by boiling the purine in hexamethylenedisilazane (20 ml per g of purine) (for Ib-d), with addition of 2 ml of trimethylchlorosilane (for Ie, f) or 2 ml of pyridine (for Ia) until complete dissolution. The solution was evaporated in vacuum, 20-30 ml of p-xylene was added, and the solution was again evaporated. The silylated purines IIa-f were used without further purification.

1-(6-Methylaminopurinyl-9)-2,5-di-O-acetyl-β-D-glucofuranurono-3,6-lactone (IVa). To a solution of 1.48 g (6.71 mmole) of 9-trimethylsilyl-6-methylaminopurine (IIa) in 100 ml of 1,2-dichloroethane was added 1.84 g (6.1 mmole) of lactone III and 1.71 g (1.38 ml; 7.32 mmole) of TMS-TF and the mixture was heated in an oil bath for 12 h at 80°. After cooling to 20° the solution was poured into a vigorously stirred suspension of sodium bicarbonate in 300 ml of chloroform and 50 ml of acetonitrile. The mixture was stirred for 1 h, the precipitate was filtered off and washed with 2 × 100 ml of chloroform, and the combined filtrates were evaporated in vacuum. The residue was dissolved in a minimal amount of chloroform, transferred to a column of 100 cm^3 of silica gel with chloroform, and eluted successively with 500 ml of chloroform and 300 ml each of 99:1 (by volume), 98:2, and 97:3 chloroform-ethanol, until all lactone IVa had come off the column. The fractions containing IVa were combined and evaporated to dryness in vacuum. The yield of analytically pure IVa was 0.35 g (15%); mp 204-205; R_f 0.42; $[\alpha]_D^{20}$ 89.2° (c 0.56, DMFA). UV spectrum: λ_{max} 265 nm (log ε 4.24). Found: C 49.6; H 4.2; N 17.6%. $C_{16}H_{17}N_5O_7$. Calculated: C 49.1; H 4.4; N 17.9%.

1-(6-Butylaminopurinyl-9)-2,5-di-O-acetyl-β-D-glucofuranurono-3,6-lacton (IVb) was obtained condensation of 9-trimethyl-silyl-6-butylaminopurine (IIb) with lactone III, similarly to IVa. After solvent was evaporated the residue was dissolved in 20 ml of chloroform, 10 cm^3 of silica gel was added, the mixture was evaporated to dryness and transferred with hexane to a column of 100 cm^3 of silica gel. Material was eluted with 500 ml of hexane, then by linear gradient elution with 500 ml each of hexane and ethyl acetate. The fractions containing IVb were combined and evaporated. The yellow oily residue was dissolved in the minimal volume of chloroform and repeatedly chromatographed on a column of 100 cm^3 silica gel with chloroform. Elution was carried out with chloroform. The fractions containing IVb were evaporated to give a frothy residue. Yield, 17%; R_f 0.54; $[\alpha]_D^{20}$ 69.3° (c 0.53, DMFA). UV spectrum: λ_{max} 267 nm (log ε 4.21). Found: C 52.6; H 5.3; N 16.1%. $C_{19}H_{23}N_5O_7$. Calculated: C 52.6; H 5.4; N 16.2%.

1-(6-Cyclohexylaminopurinyl-9)-2,5-di-O-acetyl-β-D-glucofuranurono-6,3-lactone (IVc) was synthesized similarly to IVa from 9-trimethylsilyl-6-cyclohexylaminopurine (IIc) and lactone III, and was isolated similarly to glucuronide IVb. Yield, 39%, mp 175-177°; R_f 0.53; $[\alpha]_D^{20}$ 77.1° (c 0.54; DMFA). UV spectrum: λ_{max} 267 nm (log ε 4.30). Found: C 54.8; H 5.6; N 15.0%. $C_{21}H_{25}N_5O_7$. Calculated: C 54.9; H 5.5; N 15.2%.

1-(6-Benzylaminopurinyl-9)-2,5-di-O-acetyl-β-D-glucofuranurono-6,3-lactone (IVd) was synthesized from 9-trimethylsilyl-6-benzylaminopurine (IId) and lactone III and isolated similarly to IVa. Yield, 41%; mp 11-113°; R_f 0.54; $[\alpha]_D^{20}$ 70.2° (c 0.56, DMFA). UV spectrum: λ_{max} 266 nm (log ε 4.27). Found: C 56.7; H 4.4, N 14.8%. $C_{22}H_{21}N_5O_7$. Calculated: C 56.5; H 4.5; N 15.0%.

1-(6-Morpholinopurinyl-9)-2,5-di-O-acetyl-β-D-glucofuranurono-6,3-lactone (IVe) was synthesized and isolated similarly to IVa from 9-trimethylsilyl-6-morpholinopurine (IIe) and lactone III. Yield 26%. Mp 99-101°; R_f 0.56; $[\alpha]_D^{20}$ 83.8° (c 0.56, DMFA). UV spectrum: λ_{max} 277 nm (log ε 4.28). Found: C 50.7; H 4.7; N 15.6%. $C_{19}H_{21}N_5O_8$. Calculated: C 51.0; H 4.7; N 15.6%.

1-(6-Furfurylaminopurinyl-9)-2,5-di-O-acetyl-β-D-glucofuranurono-6,3-lactone (IVf) was synthesized similarly to IVa from 9-trimethylsilyl-6-furfurylaminopurine (IIf) and lactone III, and isolated similarly to IVb. Yield 23%; mp 175-176°; R_f 0.54; $[\alpha]_D^{20}$ 85.0° (c 0.40, DMFA). UV spectrum: λ_{max} 266 nm (log ε 4.26). Found: C 52.4; H 4.1; N 15.4%. $C_{20}H_{19}N_5O_8$. Calculated: C 52.5; H 4.2; N 15.3%.

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