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CHEMICAL MODIFICATION OF BRITANIN AND OF INUCHENOLIDE C

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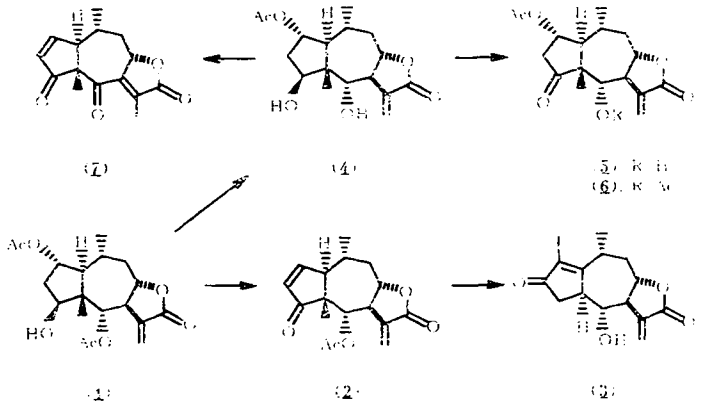
Analogs of helenalin possessing a broad spectrum of biological activity have been synthesized from the readily available sesquiterpene lactones britanin and inuchenolide C. Conditions for the hydrolysis, oxidative transformations, and skeletal rearrangements of the pseudoguaianolides that are characteristic for plants of the genus Inula *have been studied.*

Britanin (**1**), a sesquiterpene lactone that is widespread in plants of the Asteraceae family and has been isolated from *Inula caspica* Blume, *I. britanica* L., *I. aspera* Poir., and *Erigeron khorassanicus* Boiss. [1-3] is a colorless crystalline substance with mp 188-190°C, $[\alpha]_D^{20}$ −26° (*c* 5.0, chloroform).

Britanin is a close analog of the biologically active pseudoguaianolide helenalin, isolated from the American plant *Helenium autumnale* and possessing antifeedant, antileukemic, antiinflammatory, antitumoral, and cytotoxic activities [4-6]. The high biological activity of helenalin is due to the presence, together with an α-methylene-γ-lactone function, of an α,β-unsaturated cyclopentanone ring. However, the specific saturation of the C2−C3 double bond causes a manyfold decrease in the activity of the molecule.

We have carried out modification of the britanin molecule with the aim of a directed search for helenalin analogs.



The reaction of britanin (**1**) with pyridinium chlorochromate (PCC) in dichloromethane formed compound (**2**) with near-quantitative (89%) yield. The PMR spectrum (Table 1) showed the absence of a signal from one of the acetoxy groups.

The downfield shift of the signal of the protons of the angular methyl group by 0.5 ppm (s, 1.58* ppm) is due to the formation of a keto group at C-4, while the presence of specific signals of a proton at C-3 − a doublet of doublets of doublets at 7.5 ppm (1H, SSCCs 6.0 and 1.8 Hz) − and of a proton at C-2 − a doublet of doublets at 6.11 ppm (1H, SSCCs 6.0 and 2.2 Hz) − shows the formation of an olefinic double bond at these carbon atoms. In addition, signals were observed of the

*Given as 1.68 in Table 1 − Translator.

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TABLE 1. Details of the PMR Spectra of Britanin and its Derivatives (0 – TMS, C_5D_5N, δ, ppm)

Compound	Protons, chemical shifts, SSCCs, Hz											
	H-1	H-2	H-3	H-4	H-6	H-8	H-9	H-10	H-13	H-14	H-15	Other protons
1	1.35 dd	6.05 q $J_1=J_2=8.5$ $J_3=11.2$	2.32 m	3.91 dd $J_1=6.5$ $J_2=9.5$	5.11 dd $J_1=1.5$ $J_2=7.5$ $J_3=7.5$	4.5 dd $J_1=2.5$ $J_2=10$ $J_3=10.5$	2.14 m	1.78 m	6.21 dd $J_1=1.0$ $J_2=3.5$ 5.2 dd $J_1=1.0$ $J_2=3.5$	0.88 d	1.08 s	OAc 1.72 s -OAc 1.92 s
2	3.0 d.tr $J_1=2.5$ $J_2=11.0$	7.5 ddd $J_1=1.8$ $J_2=6.0$	6.11 dd $J_1=2.2$ $J_2=6.0$		5.0 d $J=3.6$	4.76 ddd $J_1=1.0$ $J_2=3.6$ $J_3=12.0$	2.35 m	1.81 m	5.97 d $J=3.5$ 6.23 d $J=2.9$	1.05 d	1.68 s	-OAc 2.0 s
3				2.90 t $J=7.8$	3.04 m	4.40 ddd $J_1=1.0$ $J_2=9.0$ $J_3=18.5$	2.47 ddd $J_1=1.2$ $J_2=6.5$ $J_3=12.0$	2.9 dd $J_1=2.2$ $J_2=8.5$	6.32 d $J=3.0$ 5.55 d $J=3.0$	0.95 d	1.69 d $J=1.0$	H-5 2.53 m
4	1.37 q	4.85 br.t $J=7.0$	2.38 m	4.57 dd $J_1=8.9$ $J_2=11.0$	3.26 d $J=3.5$	4.27 ddd $J_1=12$ $J_2=11$ $J_3=2.5$	1.85 m	1.72 m	6.11 d $J=2.5$ 6.1 d $J=3.0$	0.91 d $J=5.5$	0.89 s	-OAc 1.99 s
5	1.11 q	4.92 q $J_1=9.0$ $J_2=9.0$	1.77 dd $J_1=7.5$ $J_2=8.0$			5.12 ddd $J_1=2.0$ $J_2=8.5$ $J_3=8.5$	2.27 m	2.08 m	5.80 q $J_1=2.5$ $J_2=3.0$ 6.05 q $J_1=2.1$ $J_2=3.5$	0.88 d $J=8.0$	1.31 s	-OAc 2.02 s
6	1.13 q $J_1=J_2=12.5$ $J_3=26.0$	5.81 q $J_1=J_2=8.0$ $J_3=11.0$	1.30 dd $J_1=7.5$ $J_2=8.0$			5.16 ddd $J_1=2.0$ $J_2=8.5$ $J_3=17.0$	2.19 m	2.07 m	5.52 q $J_1=J_2=2.0$ $J_3=3.5$ 5.57 q $J_1=J_2=2.0$ $J_3=3.0$	0.88 d $J=8.0$	1.33 s	-OAc 1.95 s -OAc 2.03 s
7	1.03 m	6.02 dd $J_1=2.7$ $J_2=6.0$	7.45 dd $J_1=2.0$ $J_2=6.0$			5.48 ddq $J_1=J_3=2.0$ $J_2=12.0$	2.32 ddd $J_1=3.5$ $J_2=4.0$ $J_3=13.0$ 2.62 ddd $J_1=3.5$ $J_2=4.0$ $J_3=9.0$	2.0 m	2.14 d $J=2.0$	0.95 d $J=7.0$	1.38 s	

Note: s) singlet; d) doublet; t) triplet; q) quartet; m) multiplet; br.) broadened.

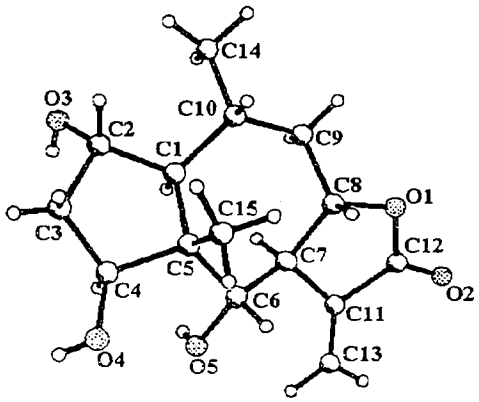


Fig. 1. Structure of the (**9**) molecule.

gem-acyl proton at C-6 – doublet at 5.00 ppm (1H, SSCC 3.6 Hz), split through interaction with a proton in the C-7 position – and the signals of the protons of the exomethylene group at C-11 in the form of two doublets at 5.97 and 6.23 ppm (1H each, SSCCs 3.5 and 2.9 Hz, respectively).

Consequently, the oxidation of the hydroxy function at C-4 in the britanin molecule is accompanied by the splitting out of the acetoxy group at C-2 and the formation of α,β-unsaturated ketobritanin (**2**).

The hydrolysis of (**2**) in ethanolic solution with a 4% solution of KOH for a day led to the neohelenalin rearrangement that has been described previously in the case of the analogous conversion of helalin into neohelenalin (mexicanin D) in [7]. In this case, the rearrangement was accompanied by migration of the double bond at C2-C3 in the (**2**) molecule into the C1-C2 position and also by the inversion of the angular methyl group at C-5 into the C-2 position. The PMR spectrum of derivative (**3**) showed a downfield shift of the signal of the C-15 protons with a change in its multiplicity – doublet at 1.69 ppm (3H, SSCC 1 Hz) – and also the absence of signals from protons at C-1 and C-2. The protons of the exomethylene group of the γ-lactone appeared in the PMR spectrum in a weaker field (than in (**2**)) in the form of two symmetrical doublets at 6.32 and 5.55 ppm (1H each, SSCC 3.0 Hz). In addition, the signal of the proton at C-5 appeared in the form of a multiplet at 2.53 ppm.

The hydrolysis of britanin (**1**) in ethanolic solution with a 4% aqueous solution of potash formed a mixture of three substances, among which deacetylbritanin (**4**) had a near-quantitative (89%) yield. Its spectrum lacked the signals of the methyl protons of one of the acetoxy groups. We also observed a change in the nature of the signals of the protons of the exomethylene group in the γ-lactone ring in the form of doublets in the 6.11 and 6.10 ppm regions, and an upfield shift by 1.58 ppm and a change in the multiplicity of the signal at C-6 to a doublet at 3.26 ppm (1H, SSCC 3.5 Hz). The signal of the protons of the angular methyl group in the form of a singlet had shifted upfield by 0.2 ppm.

The subsequent oxidation of (**4**) with chromic anhydride in dichloromethane led to the formation of the keto derivative (**5**), the PMR spectrum of which lacked the signal of a proton at C-4. Oxidation at this position was also confirmed by nature of the signals of the protons of the exomethylene group of the γ-lactone, which had not changed as a result of the reaction: two quartets at 5.80 and 6.05 ppm (1H each, SSCCs 2.5 and 3.0 Hz, and 2.1 and 3.5 Hz, respectively). We also observed an upfield shift of the signal of the proton at C-3 by 0.61 ppm: doublet of doublets at 1.77 ppm (2H, SSCCs 7.5 and 8.0 Hz).

The presence of a secondary hydroxy group in the (**5**) molecule was shown by the formation of the acetoxy derivative (**6**) when it was acylated with acetic anhydride in pyridine. The reaction took a day. The PMR spectrum of the derivative (**6**) that was formed included signals characteristic for the methyl protons of acetoxy groups: singlets at 1.55 and 2.03 ppm (3H each).

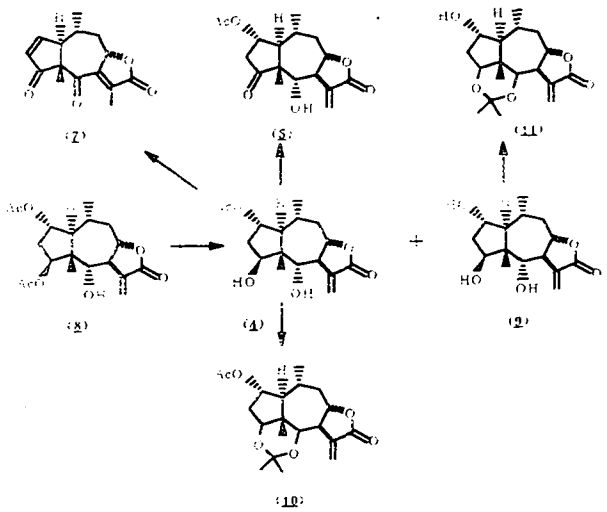
The oxidation of (**4**) with pyridinium chlorochromate in dichloromethane for 6 h formed a mixture of two substances. By the chromatographic separation of the mixture we isolated a colorless crystalline substance (**7**) the PMR spectrum of which included the signals of protons at C-4, C-6, and C-7 and also those of the protons of an exomethylene group of a γ-lactone; there were no signals of the methyl protons of an acetoxy group. At the same time, we observed a downfield shift of the signals of the angular methyl group by 0.47 ppm in comparison with that for (**4**) and by 0.27 ppm in comparison with (**5**). This was obviously caused by the formation of keto groups at C-4 and C-6 in diketobritanin (**7**). A characteristic signal of the C-13 methyl was observed – doublet at 2.14 ppm (3H, SSCC 2 Hz) – and also specific signals of the protons at C-2 and C-3

characterizing the formation of an olefinic bond – doublet of doublets at 6.02 ppm (1H, SSCCs 6.0 and 2.7 Hz) and doublet of doublets at 7.45 ppm (1H, SSCCs 6.0 and 2.0 Hz).

It must be mentioned that on the oxidation of (**4**) with chromic anhydride in pyridine only one hydroxy group, that at C-4, was oxidized, while on oxidation with pyridinium chlorochromate both hydroxy groups, at C-4 and C-6, were oxidized and, in addition, the C11-C13 double bond migrated into the C7-C11 position.

Inuchenolide C (**8**) – a sesquiterpene lactone of the pseudoguaiane type widespread in plants of the Asteraceae family – has been isolated from *Inula caspica* Blume and *I. britanica* L. The structure of the inuchenolide C molecule is close to that of britanin (**1**), differing from it by the arrangement of the OAc and OH groups at C-4 and C-6. The nature of the linkage of the rings and also the spatial structure of the molecule were determined by an x-ray structural investigation.

Reactive centers in inuchenolide C (**8**) are the OH and OAc groups, and also the lactone ring. Addition reactions of the Michael type take place mainly at the exomethylene group of the latter.



When inuchenolide C (**8**) in ethanolic solution was hydrolyzed with a 4% aqueous solution of NaOH at room temperature for 3 h, a mixture of three substances was formed. Two derivatives were isolated by the chromatographic separation of this mixture on a column of silica gel. From its spectral characteristics and physicochemical constants the first compound proved to be identical with deacetylbritanin (**4**) (see the chemical modification of britanin).

After crystallization from ethanol, the second substance (**9**) was obtained in the form of colorless crystals. The IR spectrum of dideacetylinuchenolide C (**9**) showed the absence of ester groups from its structure and the presence of –OH groups (3470, 3430, 3320 cm^{-1}). Its PMR spectrum (Table 2) lacked signals of the methyl protons of acetoxy groups. The signal of the proton at C-2 was shifted upfield by 0.8 ppm and appeared in the form of a doublet of doublets of doublets (1H, SSCCs 2.3, 6.0, and 10.0 Hz), while the proton at C-4 appeared in the 5.32 ppm region in the form of a doublet of doublets (1H, SSCCs 9.0 and 8.5 Hz). The proton at C-6 appeared in the form of a doublet in the 3.97 ppm region (1H, SSCC 9 Hz). The signals of the protons of the exomethylene group of the γ-lactone were superimposed and gave a multiplet in the 6.32 region (2H).

Four doublets at 72.85, 73.33, 76.71, and 75.48 ppm in the ^{13}C NMR spectrum showed the presence in its carbocycle of five methine fragments bound to oxygen atoms. One of these carbon atoms is linked to the lactone ring, and another three to hydroxy groups. Consequently there are three hydroxy groups in the structure of the compound obtained, dideacetylinuchenolide C (**9**).

The use of methods of two-dimensional $^1H-^1H$ NMR spectroscopy (COSY) enabled us to make an unambiguous assignment of the H-6, H-7, H-8, H-2, and H-4 signals in the NMR spectrum of (**9**) taken in C_5D_5N.

In particular, the SSCC of 9 Hz for the H-6 signal (doublet at 3.97 ppm) showed the *trans*- orientation of H-6 relative to the vicinal H-7 proton. A doublet of a doublet of doublets at 4.53 ppm was assigned to the lactone proton, the SSCCs of which characterized its *trans*- orientation relative to H-7 (J_1 = 10 Hz), its *trans*- orientation relative one of the H-9 protons (J_2 = 12 Hz), and its *cis*- orientation relative to the other H-9 proton (J_3 = 2.5 Hz). Thus, the lactone ring is located at C7–C8 and has the *trans*- linkage relative to the carbocycle of the molecule.

TABLE 2. Details of the PMR Spectrum of Inuchenolide C and Its Derivatives (0 – TMS, C_5D_5N, δ, ppm)

Compound	Protons, chemical shifts, SSCCs, Hz											
	H-1	H-2	H-3	H-4	H-6	H-8	H-9	H-10	H-13	H-14	H-15	Other protons
8	1.39 q	5.11 ddd J_1 1.5 J_2=7.5	3.12 m	6.05 dd J_1=8.0 J_2=11.5	3.91 dd J_1=6.5 J_2=9.5	1.55 ddd J_1 2.5 J_2 10.0	3.12 m	2.13 m	6.20 q J_1 1.1 J_2=3.5 6.28 q J_1 1.1 J_2=3.5	0.87 d	1.08 s	-OAc 1.77 s -OAc 1.93 s
9	2.05 dd	1.21 ddd J_1=10.0 J_2=6.0 J_3=2.3	3.27 m 3.29 m	5.32 dd J_1=9.0 J_2=8.5	3.97 d J=9.0	1.53 ddd	3.28 m	1.81 m	6.32 m	1.27 d	1.13 s	
10	1.49 dd	4.96 ddd J_1=1.5 J_2=7.5 J_3=14.5	2.09 m 1.85 ddd J_1=1.5 J_2=7.5 J_3=11.5	6.11 dd J_1=2.5 J_2=10.5	4.12 d J=7.5	1.52 ddd J_1=4.0 J_2=7.5 J_3=9.5	2.49 m	2.09 m	5.99 d J=2.9 6.21 d J=3.0	1.13 d	1.14 s	-OAc 2.0 s H2' 1.23 s H3' 1.25 s
11	1.75 q	1.7 br.t J=6.5	1.92 m 2.03 m	4.37 dd J_1=7.0 J_2=11.0	3.61 d J=9.0	1.1 ddd J_1 2.0 J_2=9.0 J_3 9.5	2.15 d.t 1.35 m	1.62 m	5.94 q J_1 1.2 J_2=3.5 6.22 q J_1 1.2 J_2=3.5	1.17 q	0.87 s	H2' 1.32 s H3' 1.37 s

Correlation of the signal of the protons with those of the carbon atoms was made by $^1H-^{13}C$ two-dimensional spectroscopy (COLOC).

The relative configurations of the hydroxy groups at C-2 and C-4 and of the methyl group at C-10, and the linkage of rings *A* and *B* in the molecule of this derivative (**9**) were established by an x-ray structural investigation (Fig. 1) [8].

The linkage of the 5- and 7-membered rings and also that of the 7-membered ring and the lactone ring are of the *trans*-type (the torsional angles H1C1C5C15 and H7C7C8H8 are 168° and 156°, respectively). The hydroxy groups at the C-2, C-4, and C-6 atoms have α-, β-, and α-orientations, respectively. The methyl groups at the C-5 and C-10 atoms are oriented in the β- and α-directions, respectively. The conformation of the 5-membered carbocycle is an almost ideal 4,5-half-chair (ΔC_2^2 = 1.4°), while the γ-lactone ring is intermediate between a 7α-envelope and a 7α,8β-half-chair (ΔC_5^7 = 5.0 and ΔC_2^{12} = 4.6°). In the dideacetylinuchenolide C (**9**) molecule, the heptane ring assumes the conformation of a considerably distorted 1,10α,8,9β-*twist*-chair (ΔC_2^6 = 16.0° and ΔC_2^9 = 28.9°). Such a considerable deviation from a pure *twist*-chair conformation is explained by steric repulsion between the C-15 Me group and the H atom at C-8.

In order to confirm the proposed structure, (**4**) was oxidized with PCC in methylene chloride. After chromatographic purification on a column of silica gel, a colorless crystalline substance was isolated which was identical, according to its spectral characteristics, with diketobritanin (**7**) (see the chemical modification of britanin). On the other hand, according TLC in ethyl acetate, the oxidation of (**4**) with CrO_3/Py in methylene chloride for 2 h formed a compound which was identified from its physicochemical properties as the keto derivative of britanin (**5**).

The interaction of (**4**) with acetone in the presence of *p*-toluenesulfonic acid led to the formation of the ketal (**10**). The PMR spectrum of compound (**10**) contained the signals of the methyl protons of a ketal group at C-2′ and C-3′ in the 1.23 ppm (singlet, 3H) and 1.25 ppm (singlet, 3H) regions. In the 4.12 ppm region was observed the signal of the proton at C-6 in the form of a doublet (1H, SSCC 7.5 Hz). The signal of the proton at C-4 was strongly shifted downfield and appeared in the 6.11 pm region in the form of a doublet of doublets (1H, SSCCs 10.5 and 2.5 Hz). There was a change in the multiplicities of the signals of the protons of the γ-lactone exomethylene group – a doublet at 5.99 ppm (1H, SSCC 2.9 Hz) and a doublet at 6.21 ppm (1H, SSCC 3.0 Hz).

The interaction of (**9**) with acetone in the presence of *p*-toluenesulfonic acid was accompanied by the formation of the ketal (**11**). The PMR spectrum of the compound obtained (**11**) contained the signals of the methyl protons of the ketal group at C-2′ and C-3′ in the form of singlets in the 1.32 and 1.37 ppm regions. The signal of the proton at C-2 appeared in the form of a broadened triplet in the 4.70 ppm region (1H, SSCC 6.5 Hz).

The signal of the proton at C-4 had shifted upfield by 0.95 ppm – a doublet of doublets (1H, SSCCs 11.0 and 7.0 Hz) – and a doublet assigned to the proton at C-6 had shifted similarly by 0.36 ppm (1H, SSCC 9.0 Hz). The protons of the γ-lactone exomethylene group appeared in the form of symmetrical quartets in the 5.94 and 6.22 ppm regions (1H each, SSCCs 3.5 and 1.2 Hz).

EXPERIMENTAL

Britanin (**1**) and inuchenolide C (**8**) were isolated from *Inula caspica* Blume by a method described previously [2]. The derivatives obtained were purified by column and flash column chromatographies. For column chromatography we used type KSK silica gel (360-630 μm) at a ratio of material to support of 1:20, the eluents being benzene containing increasing concentrations of ethyl acetate (from 0 to 100%) and acetone.

For flash column chromatography we used Amsorb brand silica gel (100-160 μm) at a ratio of material to support of 1:30, the eluent being petroleum ether with increasing concentrations of ethyl acetate (from 0 to 100%).

The individuality of the compounds was checked by thin-layer chromatography (TLC) on Silufol plates. The chromatograms were revealed with saturated $KMnO_4$ solution.

For analysis, the substances were dried in a vacuum pistol with P_2O_5 over alcohol for 6-8 h.

The elementary analyses of the compounds obtained corresponded to the calculated figures.

NMR spectra were recorded in the nuclear magnetic resonance group of NIOKh SO RAN [Novosibirsk Institute of Organic Chemistry, Siberian Division of the Russian Academy of Sciences] on Bruker WP 200SY (^1H, 200 MHz; ^{13}C, 327 MHz) and Bruker AM-400 (^1H, 400.13 MHz; ^{13}C, 100.614 MHz) instruments.

PMR spectra were obtained for solution in C_5D_5N with tetramethylsilane (TMS) as internal standard, δ-scale ^{13}C NMR spectra were recorded for solutions in $CDCl_3$ or C_5D_5N, with TMS as internal standard, δ-scale.

IR spectra were recorded on a UR-20 instrument in KBr tablets or for solutions in chloroform.

X-ray structural analysis was conducted by workers of the x-ray structural investigation [RSA] laboratory of INÉOS RAN [Institute of Heteroorganic Compounds, Russian Academy of Sciences] and the XSI group of LFMI [Laboratory of Physicomechanical Investigations], NIOKh SO RAN on Syntex P2 and Hilger-Watts diffractometers using Mo radiation with a graphite monochromator.

Keto Derivative of Britanin (2). With stirring, 8 g of PCC was added to a solution of 750 mg (**1**) in 10 ml of CH_2Cl_2, and the mixture was left overnight at room temperature. TLC then showed a single spot. The reaction mixture was treated with 10 ml of water and was extracted three times with chloroform (3 × 100 ml). After acidification with 10% HCl to pH 1 the extract was washed with saturated solutions of $NaHCO_3$ and NaCl to pH 7. It was dried over $MgSO_4$, and the solvent was distilled off in a rotary evaporator under vacuum. The yield of crude product was 1.3 g. It was purified by flash chromatography on a column. The eluent used was a mixture of hexane and ethyl acetate (7:3). The product was recrystallized from hexane–ethyl acetate (1:1), giving a colorless crystalline substance with the composition $C_{17}H_{20}O_5$, mp 178-181°C. Yield 563 mg (89%), R_f 0.8 (TLC in ethyl acetate).

Hydrolysis of Britanin (1). With stirring, 6 ml of 4% K_2CO_3 (pH 10) was added to a solution of britanin (**1**) in 10 ml of EtOH. After 6 h, the reaction mixture was acidified with 3% HCl to pH 1. TLC showed three spots: R_f 0.83 (traces of britanin), 0.64 (**4**), 0.37 (traces of the dideacetyl derivative). It was extracted with ethyl acetate, and the extract was washed with saturated solutions of $NaHCO_3$ and NaCl to pH 7 and was dried over $MgSO_4$ and evaporated. The yield of crude product was 220 mg. It was separated by flash column chromatography, the desired product being eluted with hexane–ethyl acetate (7:3). After recrystallization from hexane–ethyl acetate (1:1) a colorless crystalline substance was obtained with the composition $C_{17}H_{24}O_6$, mp 98-101°C. Yield 174 mg (89%).

Chromic Anhydride Oxidation of (4). With stirring, a sixfold excess of CrO_3/Py was added to a solution of 470 mg of (**4**) in 10 ml of CH_2Cl_2. After 2 h, TLC showed the formation of product (**5**) with R_f 0.8. The reaction mixture was subjected to the usual treatment and the solvent was evaporated off, and then chromatographic purification with elution by hexane–ethyl acetate (4:1) yielded the desired product, which, after recrystallization from hexane–ethyl acetate (1:1) consisted of a colorless crystalline substance with the composition $C_{17}H_{22}O_6$, mp 223-225°C. Yield 210 mg (50%).

Elution with hexane–ethyl acetate (3:2) led to the isolation of trace amounts of the starting material.

Acylation of (5). A solution of 731 mg of (**5**) in 3 ml of pyridine was treated with 2 ml of acetic anhydride. After an hour, the reaction mixture was worked up in the usual way. TLC showed the formation of a product with R_f 0.9 (**6**) and the presence of trace amounts of the starting material. After evaporation, the crude product (800 mg) was chromatographed on a column.

Elution with hexane–ethyl acetate (6:1) led to the isolation of (**6**). After recrystallization from alcohol a colorless crystalline substance was obtained with the composition $C_{19}H_{24}O_7$, mp 210-212°C. Yield 236 mg (38%).

Pyridinium Chlorochromate Oxidation of (4). With stirring, 2 g of PCC was added to a solution of 180 mg of (**4**) in 15 ml of CH_2Cl_2. After 6 h, TLC showed the formation of two products, with R_f 0.8 and 0.75. Following the usual working up of the reaction mixture and evaporation of the solvent, the crude product (106 mg) was separated by flash column chromatography with elution by hexane–ethyl acetate (7:3). The first product, in trace amounts, could not be isolated. The second derivative (**7**) was recrystallized from ethanol, giving a colorless crystalline substance with the composition $C_{15}H_{16}O_4$, mp 170-175°C. Yield 25 mg (14%), R_{f1} 0.8 and R_{f2} 0.7 (TLC in ethyl acetate).

Hydrolysis of (2). With stirring and heating to 40°C, 152 mg of (**2**) was dissolved in 25 ml of ethanol, and the pH was brought to 9-9.5 by the addition of 4% aqueous KOH. The reaction mixture was left for a day at room temperature and was then acidified to pH 1 with 15% HCl. A TLC plate showed two spots: R_f 0.8 (**2**) and 0.56 (**3**). After chloroform extraction, the extract was washed with saturated $NaHCO_3$ and NaCl to pH 7, dried over $MgSO_4$, and evaporated. The yield of crude product was 156 mg. It was separated by flash column chromatography with elution by hexane–ethyl acetate (3:2). Product (**3**) was recrystallized from ethanol, giving a colorless crystalline substance with the composition $C_{15}H_{18}O_4$, mp 250-255°C. Yield 100 mg (70%).

Hydrolysis of Inuchenolide C (8). With stirring, 4% aqueous NaOH was added to a solution of 1.28 g of inuchenolide C in 10 ml of ethanol to bring the pH to 9-9.5. After 3 h, the reaction mixture was acified to pH 1.

On a TLC plate there were three spots: R_{f1} 0.75 (starting material), R_{f2} 0.55, and R_{f3} 0.2 (ethyl acetate). The reaction mixture was washed with saturated solutions of $NaHCO_3$ and NaCl to pH 7 and was extracted with chloroform (3 × 100 ml). The chloroform extract was dried over Na_2SO_4 and the solvent was evaporated off. According to TLC, part of the reaction

product was present in the aqueous fraction. This was evaporated, and the combined residues were chromatographed on a column of type KSK silica gel at a ratio of material to sorbent of 1:20.

After chromatographic separation and crystallization, product (**4**) consisted of a colorless crystalline substance with R_f 0.55, composition $C_{17}H_{24}O_6$, mp 98-101°C. IR spectrum: 3500, 1780, 1750, 1675 cm^{-1}.

The third product, (**9**), with R_f 0.2, was a colorless crystalline substance, $C_{15}H_{22}O_5$, mp 177-178°C (ethanol). IR spectrum: 3740, 3430, 3320, 1745, 1670 cm^{-1}. Mass spectrum (*m/z*, intensity, %): 283 (M^+ 1.7%), 264 ($M^+ - H_2O$), 246 ($M^+ - 2H_2O$), 228 ($M^+ - 3H_2O$).

Pyridinium Chlorochromate Oxidation of (4). With stirring, 1.6 g of PCC was added a solution of 100 mg of (**4**) in 10 ml of CH_2Cl_2, and the mixture was left overnight. A TLC plate showed the formation of a product with R_f 0.75 (ethyl acetate). After working up and purification on a column of silica gel, the product obtained (**7**) was recrystallized from ethanol. Colorless crystalline substance with the composition $C_{15}H_{16}O_{46}$, mp 170-175°C. Yield 20 mg (17%).

Chromic Anhydride Oxidation of (4). With stirring, a sixfold excess of CrO_3/Py was added to a solution of 350 mg of (**4**) in 10 ml of CH_2Cl_2. After 2.5 h, the formation of compound (**5**) was revealed on a TLC plate in the ethyl acetate system, R_f 0.8. The product was worked up and was purified on a column of silica gel and was then recrystallized from hexane–ethyl acetate (1:1). Colorless crystalline substance with the composition $C_{17}H_{22}O_6$, mp 223-225°C. Yield 150 mg (50%).

Synthesis of the Ketal of Monodeacetylinuchenolide C (10). A solution of 440 mg of (**4**) in 10 ml of acetone was treated with 30 ml of benzene and 20 mg of *p*-TsOH. The reaction mixture was boiled with a Dean-Stark trap for 10 h. TLC in the hexane–ethyl acetate (1:1) system showed the formation of product (**10**). After purification on a column of silica gel and recrystallization from alcohol, a substance was isolated with the composition $C_{20}H_{28}O_6$, mp 118-121°C.

Synthesis of the Ketal of Dideacetylinuchenolide C (11). A solution of 504 mg of dideacetylinuchenolide C (**9**) in 20 ml of acetone was treated with 50 ml of benzene and 30 mg of *p*-TsOH, and the reaction mixture was boiled with a Dean-Stark trap for 5.5 h. TLC in the hexane–ethyl acetate (1:1) system showed the formation of a product with R_f 0.72.

The reaction mixture was washed with water, and the aqueous layer was separated off and neutralized with $NaHCO_3$ solution. The organic layer was washed again with the same aqueous fraction, being brought to a neutral reaction, and the solvent was distilled off. After purification on a column of silica gel, the product obtained was crystallized from alcohol. Yield 98 mg (23%).

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