ISOPRENOIDS OF VARIOUS SPECIES OF THE GENUS Betula

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The review contains an analysis of the literature up to 1986, inclusive on the chemical investigation of some species of the genus Betula (family Betulaceae). Attention is devoted mainly to the distribution of the triterpene compounds in various parts of birch trees, and their biological properties and prospects are also discussed. Information (structure, melting point, angle of rotation, source) is given on 33 triterpenoids of the dammarane series isolated from the leaves, catkins, and young twigs of birch trees, and of 23 triterpenoids of the lupane and β-amyrin series isolated from the outer bark.

About 100 species of the genus Betula (family Betulaceae) grow on the territory of the Soviet Union [1]. The number of species has not been established accurately because of the existence of numerous hybrid forms which are frequently described as independent species. Furthermore, polymorphism is characteristic for all species of birch, and this further complicates the already confused systematics of the genus Betula [2]. Valuable timber-forming species are most widely distributed in the USSR: the common or European white birch (Betula verrucosa Ehrh. = Betula pendula Roth.) and Betula pubescens Ehrh. [~ downy birch] [3].

The birch may serve as a source of various extractive substances. A wide range of methylated flavones and flavonols have been found in extracts of birch buds [4]. Birch seeds from a rich source of fatty acids, especially linoleic [5]. Birch leaves contain flavonoids, anthocyans, coumarins, tanning substances, organic acids, carotenoids, carbohydrates, essential oils, and resins [6]. Birch bark is distinguished by a particularly high concentration of extractive substances which include mono- and triterpenoids, carbohydrates, alcohols, fatty and resin acids, and phenolic compounds [7]. The presence and amounts of representatives of these classes of organic compounds are, as a rule, determined by the phase of development of the tree.

The present review is devoted to triterpenoids of the dammarane, lupane, and β-amyrin series from extracts of the leaves, catkins, twigs, and bark of the birch. A review published in 1984 on secondary metabolites of the genera Betula, Salix, and Populus contains incomplete and not totally reliable information on the presence of triterpenoids in birch extracts [8].

TRITERPENOIDS OF THE DAMMARANE SERIES

Triterpenoids of the dammarane series were first isolated from birch leaves (Betula pendula Roth.) in 1959 by the German chemists Fischer and Seiler [9]. They showed that triterpene alcohols were present in the leaf epiderm in the form of esters. The triterpenoids were isolated from the unsaponifiable fraction after preliminary treatment of an ethereal extract of the leaves with an alcoholic solution of alkali. They named the two triterpene alcohols isolated betulafolienetriol and betulafolienetetraol. The structures of these compounds were established by classical degradative methods using IR and UV spectroscopy.

These alcohols are of interest primarily because of their relationship to the aglycons of the ginseng glycosides (panaxosides or ginsenosides) which are responsible for the specific biological activity of bark extracts. Betulafolienetriol (7) differs from protopanaxadiol — the native genin of ginsenosides Rb_1, Rb_2, Rc, and Rd — only by the configuration of the hydroxy group at C-3 [10]. In a clinical study of the sapogenins of ginseng by a group of Japanese chemists under the leadership of O. Tanaka, betulafolienetriol was synthesized from hydroxyhopanone as the starting material. Betulafolienetriol is fairly easily converted

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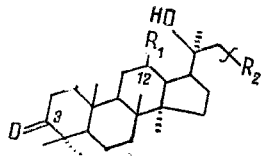
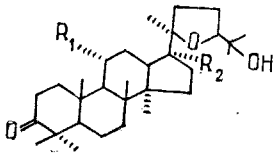
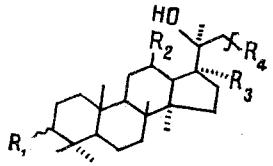
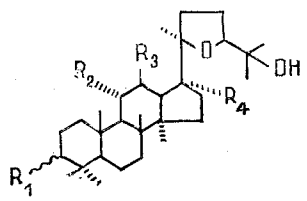
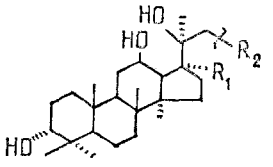
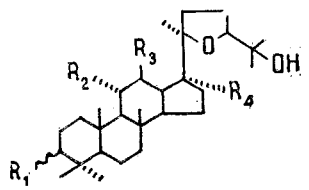
TABLE 1. Triterpenoids of the Dammarane Series

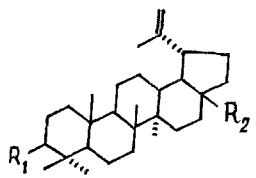
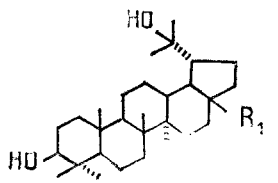
Name and molecular formula	Structure	mp, °C; $[\alpha]_D$ (solvent)	Source, literature
1. 12β, 20(S)-Dihydroxydammar-24-en-3-one $C_{30}H_{50}O_3$	 R_1 = OH; R_2 = — CH_2 — CH = C $(CH_3)_2$	196 — 199 (acetone)	Leaves of B. pendula Roth. [16], B. platyphylla Sukacz. [17], B. platyphylla Suk. var. japonica Hara [18], B. mandshurica (Regel) Nakai [19]
2. 20(S), 24(S)-Dihydroxydammar-25-en-3-one $C_{30}H_{50}O_3$	R_1 = H; R_2 = — CH_2 — CH(OH) — C(CH_3) = CH_2	216 — 218 (petroleum ether—acetone) +44 (chloroform)	Leaves of B. mandshurica (Regel) Nakai [19], B. pendula Roth. [20], B. ovalifolia Rupr. [21]
3. 12β, 20(S), 25-Trihydroxydammar-23-en-3-one $C_{30}H_{50}O_3$	R_1 = OH; R_2 = — CH = CH — C(CH_3)(OH) — CH_3	Noncryst. + 32.2 (chloroform)	Leaves of B. pendula Roth. [16]
4. 11α,25-Dihydroxy-20(S),24(R)-epoxydammaran-3-one $C_{30}H_{50}O_4$	 R_1=OH; R_2=H	90 — 97 (acetone) + 75,4 (chloroform)	Leaves of B. lanata (Regel) V. Vassil. [22]
5. 17α,25-Dihydroxy-20(S),24(R)-epoxydammaran-3-one $C_{30}H_{50}O_4$	R_1 = H; R_2 = OH	179 — 182 (hexane—acetone) +58,3 (chloroform)	Leaves of B. ovalifolia Rupr. [21]
6. Dammar-24-ene-3α, 17α, 20(S)-triol $C_{30}H_{52}O_3$	 R_1 = α-OH; R_2 = H; R_3 = OH; R_4 = — CH_2 — CH = C $(CH_3)_2$	140 — 142 (petroleum ether) + 4,8 (chloroform)	Leaves of B. pendula Roth. [16, 20], B. costata Trautw. [23]
7. Betulafolienetriol $C_{30}H_{52}O_3$	R_1=α-OH; R_2=OH; R_3=H; R_4 = — CH_2 — CH = C $(CH_3)_2$	196 — 197 (acetone) + 12,0 (chloroform)	Leaves of B. pendula Roth. [9, 16], B. fruticosa Pall. [17], B. platyphylla Sukacz. [17], B. platyphylla Suk. var. japonica Hara [18], B. mandshurica (Regel) Nakai [19], B. humilis Schrank. [20], B. middendorffii Trautv. et Mey [21], B. costata Trautw. [23], B. exilis Sukacz. [24], B. divaricata Ledeb. [25], B. dahurica Pall. [26]

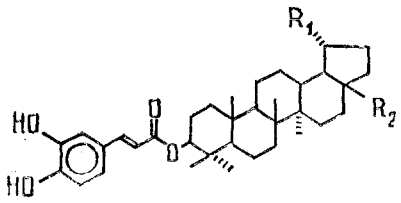
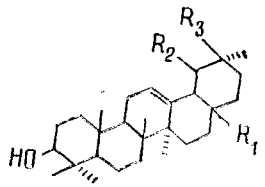
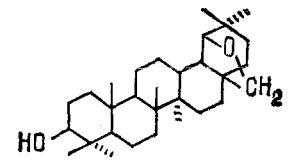
8. 3-Epiisofouquierol $C_{30}H_{52}O_3$	$R_1 = \alpha$-OH; $R_2 = R_3 = H$; $R_4 = -CH=CH-C(CH_3)_2OH$	162 — 165 (acetone) + 11,6 (chloroform)	Leaves of B. exilis Sukacz. [24]
9. Dammar-25-ene-3β, 20(S), 24ξ-triol $C_{30}H_{52}O_3$	$R_1 = \beta$-OH; $R_2 = R_3 = H$; $R_4 = -CH_2-CHOH-C(CH_3)=CH_2$		Leaves of B. exilis Sukacz. [24]
10. Dammar-25-ene-3α, (20S),24ξ-triol $C_{30}H_{52}O_3$	$R_1 = \alpha$-OH; $R_2 = R_3 = H$; $R_4 = -CH_2-CHOH-C(CH_3)=CH_2$	152 — 155 (acetone) + 22,2 (chloroform)	Leaves of B. exilis Sukacz. [24]
11. 3-Epiocotillol $C_{30}H_{52}O_3$		167 — 169 (acetone) + 19,46 (chloroform)	Leaves of B. ovalifolia Rupr. [21], B. lanata (Regel) V. Vassil. [22], B. exilis Sukacz. [24], B. nana L. [24], B. latifolia [27]; female catkins of B. exilis Sukacz. [28]; pollen of male catkins of B. platyphylla Suk. var. japonica Hara [29]
	$R_1 = \alpha$-OH; $R_2 = R_3 = R_4 = H$		
12. Ocotillol $C_{30}H_{52}O_3$	$R_1 = \beta$-OH; $R_2 = R_3 = R_4 = H$	196 — 198 (acetone) + 35,2 (chloroform)	Leaves of B. exilis Sukacz. [24] B. nana L. [24]; pollen of male catkins of B. platyphylla Suk. var. japonica Hara [29]
13. 20(S),24(R)-Epoxy-dammarane-3β, 11α, 25-triol $C_{30}H_{52}O_4$	$R_1 = \beta$-OH; $R_2 = OH$; $R_3 = R_4 = H$	216 — 218 (petroleum ether) + 24.4 (chloroform)	Leaves of B. fruticosa Pall. [17], B. ermanii Cham. [30]
14. 20(S),24(R)-Epoxy-dammarane-3α, 11α, 25-triol $C_{30}H_{52}O_4$	$R_1 = \alpha$-OH; $R_2 = OH$; $R_3 = R_4 = H$	159 — 160 (hexane) + 15,6 (chloroform)	Leaves of B. fruticosa Pall. [17], B. lanata (Regel) V. Vassil [22]
15. 20(S),24(R)-Epoxy-dammarane-3α,17α, 25-triol $C_{30}H_{52}O_4$	$R_1 = \alpha$-OH; $R_2 = R_3 = H$; $R_4 = OH$	191 — 193 (hexane—acetone + 9,0 (chloroform)	Leaves of B. pendula Roth. [16, 20], B. platyphylla Sukacz. [17], B. fusca Pall. ex Georgi [20], B. ovalifolia Rupr. [21]
16. Betulafolienetriol oxide $C_{30}H_{52}O_4$	$R_1 = \alpha$-OH; $R_2 = R_4 = H$; $R_3 = OH$	237 — 240 (acetone) + 2,6 (chloroform)	Pollen of the male catkins and leaves of B. platyphylla Suk. var. japonica Hara [18, 29]; листья B. pendula Roth. [16], B. fruticosa Pall. [17], B. platyphylla Sukacz. [17], B. mandshurica (Regel) Nakai [19], B. humilis Schrank. [20], B. fusca Pall. ex Georgi [20], B. ovalifolia Rupr. [21], B. middendorffii Trautw. et Mey [21], B. exilis Sukacz. [24], B. nana L. [24], B. divaricata Ledeb. [25], B. davurica Pall. [26]
17. Pyxitiol $C_{30}H_{52}O_4$	$R_1 = \beta$-OH; $R_2 = R_4 = H$; $R_3 = OH$	218 — 220 (acetone)	Leaves of B. humilis Schrank. [20], B. fusca Pall. ex Georgi [20]
18. Betulafolienetetraol $C_{30}H_{52}O_4$		168 — 170 (acetone + 8,5 (chloroform)	Leaves of B. pendula Roth. [9, 16], B. costata Trautw. [23], B. platyphylla Suk. var. mandshurica [31]

Name and molecular formula	Structure	mp, °C; $[\alpha]_D$ (solvent)	Source, literature
19. Dammar-23-ene-3α,12β,20(S),25-tetrasol $C_{30}H_{52}O_4$	R_1 = OH; R_2 = — CH_2 — CH = C $(CH_3)_2$ R_1 = H; R_2 = — CH = CH — C(CH$_3$)$_2$ — OH	130 — 133 (chloroform) — 4,9 (chloroform)	Leaves of B. pendula Roth. [16], B. middendorffii Trautv. et Mey [21], B. platyphylla Suk. var. japonica Hara [32]
20. Dammar-25-ene-3α,12β,20(S),24(R)-tetraol $C_{30}H_{52}O_4$	R_1 = H; R_2 = — CH_2 — COH(H) — C(CH$_3$) = CH_2	134 — 136 (acetone) + 16,3 (chloroform)	Leaves of B. pendula Roth. [16], B. platyphylla Sukacz. [17], B. humilis Schrank. [20], B. middendorffii Trautv. et Mey [21], B. exilis Sukacz. [24], B. divaricata Ledeb. [25], B. platyphylla var. mandshurica [31], B. platyphylla Suk. var. japonica Hara [32]
21. Dammar-25-ene-3α-12β,10(S),24(S)-tetraol $C_{30}H_{52}O_4$	R_1 = H; R_2 = — CH_2 — CH(OH) — C(CH$_3$) — CH_2	169 — 172 (ethanol) — 4,0 (chloroform)	Leaves of B. pendula Roth. [16], B. divaricata Ledeb. [25]
22. Betulafolienepentaol $C_{30}H_{52}O_5$	R_1 = OH; R_2 = — CH = CH — C(CH$_3$)$_2$ — OH	203 — 204 (methanol)	Leaves of B. platyphylla var. mandshurica [31]
23. Dammar-25-en-3α, 12β,17α,20(S),24ξ-pentaol $C_{30}H_{52}O_5$	R_1 = OH; R_2 = — CH_2 — CH (OH) — C(CH$_3$) = CH_2	Noncryst. — 3,0 (chloroform)	Leaves of B. pendula Roth. [16]
24. Betulafolienetetraol oxide $C_{30}H_{52}O_5$		250 — 251,5 (acetone) + 6,0 (chloroform)	Leaves of B. ovalifolia Rupr. [21], B. costata Trautw. [23]
25. 20(S),24(R)-Epoxydammarane-3β,12β,17α,25-tetraol $C_{30}H_{52}O_5$	R_1 = α-OH; R_2 = H; R_3 = R_4 = OH R_1 = β-OH; R_2 = H; R_3 = R_4 = OH	230 — 231 (petroleum ether—acetone) + 13,5 (chloroform)	Leaves of B. fusca Pall. ex Georgi [20]
26. 3α-Acetoxy-20(S),24(R)-epoxydammarane-11α,25-diol $C_{32}H_{54}O_5$	R_1 = α-OAc; R_2 = OH; R_3 = R_4 = H	198 — 200 (hexane—acetone) — 6,2 (chloroform)	Leaves of B. lanata (Regel) V. Vassil. [22]
27. 11α-Acetoxy-20(S),24(R)-epoxydammarane-3α,25-diol $C_{32}H_{54}O_5$	R_1 = α-OH; R_2 = OAc; R_3 = R_4 = H	176—178 (hexane—acetone) + 5,3 (chloroform)	Leaves of B. lanata (Regel) V. Vassil. [22]
28. 11α-Acetoxy-20(S),24(R)-epoxydammarane-3β,25-diol $C_{32}H_{54}O_5$	R_1 = β-OH; R_2 = OAc; R_3 = R_4 = H	199—201 (hexane—acetone) + 14,8 (chloroform)	Leaves of B. ermanii Cham. [30]

29. 12β-Acetoxy-20(S), 24(R)-epoxydammarane-3α-25-diol $C_{32}H_{54}O_5$	R_1 = α-OH; R_2 = R_4 = H; R_3 = OAc	139—145 (hexane) —7,6 (chloroform)	Leaves of B. platyphylla Sukacz. [17], B. nana L. [24]
30. 3α,12β-Diacetoxy-20(S), 24(R)-epoxydammarane-25-ol $C_{34}H_{56}O_6$	R_1 = α-OAc; R_2 = R_4 = H; R_3 = OAc	178—181 (hexane—acetone)	Leaves of B. nana L. [24]
31. 3-Epiocotillol 3α-malonate $C_{33}H_{54}O_6$	R_1 = α-OCO—CH_2—COOH; R_2 = R_3 = R_4 = H	161—166 (hexane—acetone) —3,8 (chloroform)	Female catkins of B. exilis Sukacz. [28]
32. Betulafolienetriol oxide 3α-malonate $C_{33}H_{54}O_7$	R_1 = α-OCO—CH_2—COOH; R_2 = R_4 = H; R_3 = OH		Female catkins of B. exilis Sukacz. [28]
33. Papyriferic acid $C_{35}H_{56}O_8$	R_1 = α-OCO—CH_2—COOH; R_2 = R_4 = H; R_3 = OAc	203—204 (acetone—cyclohexane) —18,0 (chloroform)	Female catkins of B. divaricata Ledeb. [25], B. exilis Sukacz. [28]; shoots of the current year from B. papyrifera Marsh subsp. humilis (Regel) Hult. [33], B. dahurica Pall. [34]

TABLE 2. Triterpenoids of the Lupane and β-Amyrin Series

Name and molecular formula	Structure	mp, °C; $[\alpha]_D$ (solvent)	Source, literature
36. Betulone aldehyde $C_{30}H_{46}O_2$	 $R_1 = O$; $R_2 = CHO$	164 — 166 (methanol) + 52,4	B. pubescens Ehrh. [7], B. mandshurica (Regel) Nakai [61, 62], B. pendula Roth. [63]
37. Lupenone $C_{30}H_{48}O$	$R_1 = O$; $R_2 = CH_3$	166 — 168 (methanol) + 60,6 (chloroform)	B. lenta L. [7], B. alleghanienses Britton [7], B. verrucosa Ehrh. [64]
38. Betulin aldehyde $C_{30}H_{48}O_2$	$R_1 = \beta$-OH; $R_2 = CHO$	192 — 193 (ethanol)	B. pendula Roth. [63], B. verrucosa Ehrh. [64]
39. Betulinic aldehyde $C_{30}H_{48}O_3$	$R_1 = \beta$-OH; $R_2 = COOH$	316 — 318 (ethanol) + 7,89 (pyridine)	B. verrucosa Ehrh. [7], B. pubescens Ehrh. [7], B. papyrifera Marsh. [7], B. costata Trautw. [62], B. pendula Roth. [63], B. maximowicziana Regel [65]
40. Lupeol $C_{30}H_{50}O$	$R_1 = \beta$-OH; $R_2 = CH_3$	215—216 (methanol—acetone)	B. pubescens Ehrh. [7], B. papyrifera Marsh. [7], B. lenta L. [7], B. alleghanienses Britton [7], B. mandshurica (Regel) Nakai [61, 62], B. costata Trautw. [62], B. pendula Roth. [7, 63], B. verrucosa Ehrh. [7, 64], B. maximowicziana Regel [65], B. utilis D. Don [66], B. davurica Pall. [62, 67], B. platyphylla Suk. var. japonica Hara [68].
11. Betulin $C_{30}H_{50}O_2$	$R_1 = \beta$-OH; $R_2 = CH_2OH$	251 — 252 (ethanol) + 19,96 (pyridine)	B. pubescens Ehrh. [7], B. papyrifera Marsh. [7], B. lenta L. [7], B. alleghanienses Britton [7], B. mandshurica (Regel) Nakai [61, 62], B. costata Trautw. [62], B. pendula Roth. [7, 63], B. verrucosa Ehrh. [7, 64], B. maximowicziana Regel [65], B. utilis D. Don [66], B. platyphylla Suk. var. japonica Hara [68], B. davurica Pall. [62, 67, 69]
42. Methyl betulinate $C_{31}H_{50}O_3$	$R_1 = \beta$-OH; $R_2 = COOCH_3$	223—224 +8,01 (chloroform)	B. verrucosa Ehrh. [64]
43. Monogynol A (lupane-3β,20-diol) $C_{30}H_{52}O_2$	 $R = CH_3$	233—238 (methanol—acetone) + 24,0 (chloroform)	B. verrucosa Ehrh. [64, 70], B. maximowicziana Regel [65]

44. Lupane-3β,20-28-triol $C_{30}H_{32}O_3$	R = CH_2OH	240 — 245 (acetone)	B. verrucosa Ehrh. [64, 70], B. maximowicziana Regel [65]
45. Pyracrenic acid $C_{39}H_{54}O_6$	 $R_1 = C(CH_3) = CH_2$; $R_2 = COOH$	310 — 312 (decomp., hexane—ethanol)	B. maximowicziana Regel [65], B. platyphylla Suk. var, japonica Hara [68]
46. Betulin caffeate $C_{39}H_{56}O_5$	$R_1 = C(CH_3) = CH_2$; $R_2 = CH_2OH$	Acetate: 204 — 206 (ethanol)	B. mandshurica (Regel) Nakai [61, 62], B. costata Trautw. [62], B. maximowicziana Regel [65], B. platyphylla Suk. var. japonica Hara [68], B. verrucosa Ehrh. [71]
47. Lupane-3β,20,28-triol caffeate $C_{39}H_{58}O_6$	$R_1 = COH(CH_3)_2$; $R_2 = CH_2OH$	Acetate: 120 — 125 (ethanol)	B. maximowicziana Regel [65]
48. Oleanolic acid aldehyde $C_{30}H_{48}O_2$	 $R_1 = CHO$; $R_2 = H$; $R_3 = CH_3$	169 — 172 (methanol) + 72,0 (chloroform)	B. verrucosa Ehrh. [64]
49. Oleanolic acid $C_{30}H_{48}O_3$	$R_1 = COOH$; $R_2 = H$; $R_3 = CH_3$	306 — 308 (ethanol) + 79,5 (chloroform)	B. papyrifera Marsh. [7], B. mandshurica (Regel) Nakai [61, 62], B. costata Trautw. [62], B. pendula Roth. [63], B. utilis D. Don [66], B. dahurica Pall. [62, 67, 69], B. platyphylla Suk. var. japonica Hara [68]
50. Ursolic acid $C_{30}H_{48}O_3$	$R_1 = COOH$; $R_2 = CH_3$; $R_3 = H$	284 — 285 (ethanol) + 68,0 (methanol)	B. pubescens Ehrh. [7], B. pendula Roth. [63]
51. β-Amyrin $C_{30}H_{50}O$	$R_1 = R_3 = CH_3$; $R_2 = H$	197 — 200 (ethanol) + 88,0 (chloroform)	B. verrucosa Ehrh. [64]
52. Erythrodiol $C_{30}H_{50}O_2$	 $R_1 = CH_2OH$; $R_2 = H$; $R_3 = CH_3$	236 — 237 (ethanol) + 77,0 (chloroform)	B. verrucosa Ehrh. [64]

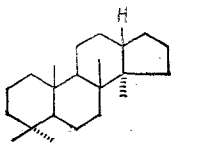
into protopanaxadiol after oxidation followed by reduction with sodium tetrahydroborate [11-13]. Triterpenoids from beech leaves have been used as the starting materials for the synthesis of physiologically active analogs of ginseng glycosides [10, 14, 15].

Later, in a study of extracts of other birch species, in addition to betulafolienetriol and betulafolienetetraol (18), a number of other dammarane triterpenes characteristic of the genus Betula were isolated. Information on all these compounds is given in Table 1. As can be seen from this table, dammarane triterpenoids are present not only in the leaves but also in the young twigs and catkins of the birch, i.e., in the other components of the tree verdure. In contrast to leaf extracts, extracts of birch catkins and twigs were not subjected to alkaline hydrolysis, which enabled the native compounds (31), (32), and (33), which are esters of triterpenoids with malonic and acetic acids, to be isolated [25, 28, 33, 34].

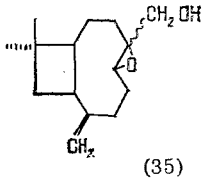
The triterpenoids of the dammarane series presented in Table 1 have not, as a rule, been detected in other plants. The only exceptions are ocotillol (12) and pyxinol (17), which were first isolated from, respectively, Fouquieria splendens Engelm. (Fouquieriaceae) [35] and Pyxine endochrysina Nyl. (Physeiaceae) [36].

It must be mentioned that the maximum amount of triterpenoids in birch leaves is present in the first half of the year and may reach 0.8% of the weight of the air-dry leaves [9, 26].

In establishing the structures of the compounds isolated, wide use has been made of physical methods (IR, mass, and ^{1}H and ^{13}C NMR spectra, X-ray structural analysis), which permit a rapid and reliable determination of the structures of triterpenoids, including minor components of an extract. One of the most informative methods for solving structural problems is ^{13}C NMR, which was first used for the triterpenoids of the dammarane series in 1977 [37]. In order to calculate the substituent effects in the ^{13}C NMR spectra of triterpenoids of the dammarane series, octanor-13β-dammarane (34) was synthesized from compound (15) [38]. This hydrocarbon (34) was used to determine the effects of side chains at C-17 on the ^{13}C shifts of the dammarane triterpenes [39].



(34)



(35)

The ^{13}C NMR method has also been used for solving stereochemical problems, and, namely, for establishing the C-24 configurations of the asymmetric centers in the open side chain of 24-hydroxy derivatives of tetracyclic triterpenes of the dammarane series [19]. It has been observed that the C-22 and C-24 signals in the spectra of compounds of the 24(S) series appear in a weaker field than the signals of the corresponding atoms for compounds of the 24(R) series. The structure with the S-configuration of carbon atom 24 for triterpene (2) has been confirmed by the results of x-ray structural analysis [40]. The C-24 configurations in the epimeric triterpenoids (20) and (21) were determined by the use of this diagnostic criterion [25]. The same approach has been used to assign the signals in the spectra in the triterpenoids (9), (10), and (23), which are irresolvable mixtures of epimers at C-24 [16, 24].

The method of X-ray structural analysis has been brought in for determining stereochemical features of the triterpenoids (5), (12), (16), (24), (28), and (33) [18, 40, 41]. In this way the configurations of the C-20 and C-24 asymmetric centers in compounds having side chains in the form of substituted tetrahydrofuran rings were demonstrated and the orientation of the hydroxy group at C-17 in compound (24) was also established.

The set of triterpenoids in the leaves is basically stable and characteristic for each species, which creates the possibility of using them for the classification of birches. However, as has been shown for the case of B. pendula Roth., the chemical composition of the leaves of the most common species within a given area may vary according to the growth site, and this must be taken into account in systematics [16]. Triterpenoids of the dammarane series have been used for the comparative taxonomic study of Far Eastern species of the genus Betula, where they served as independent chemical criteria at various taxonomic level (subgenus, section, series, species, subspecies) [42, 43].

In this connection, it is interesting to note that the leaves of some birch species contain no triterpenoids. Of the species studied at the present time, dammarane triterpenoids are absent from the leaves of B. schmidtii, which differs considerably from all known species of the genus Betula by combining characteristics of birch and alder [42], and also from the leaves of B. pubescens collected from various sites and of species and varieties close to it (B. tortuosa, B. procurva, and B. pubescens rhombifolia, [44, 45]. Methylated flavonoids and the sesquiterpene alcohol 14-hydroxycaryophyllene 4,5-oxide (35) have been found in the unsaponifiable fraction of an ethereal extract of Betula pubescens [20, 44]. This set of compounds is more charateristic for exracts of birch buds, which contain flavonoids and sesquiterpene alcohols similar to caryophyllene, the so-called betulinols [4, 46-48].

The densitometric measurement of thin-layer chromatograms has been used for the quantitative estimation of some of the main triterpenoids in the unsaponifiable fraction of an etheral extract of leaves [26]. Attempts have ben made to use ^{13}C NMR spectroscopy for the qualitative and quantitative analysis of extracts [49]. However, successful analysis has proved to be possible only for fairly narrow fractions of the total extracts. The GLC method is suitable for the rapid evaluation of a raw material for its triterpenoid composition and in the search for promising sources [50-52].

There is no information in the literature relative to the pharmacological activity of individual triterpenoids isolated from birch leaves. Birch leaves are used in medicine in the form of infusions, decoctions, and tinctures as diuretic and sudorific agents [6, 53, 54]. Aqueous extracts from birch leaves possess effective cholagogic and antigiardiasis actions [55]. In a study of the pharmacological properties of decoctions from birch leaves they were compared with a tincture of ginseng root and a tincture of ginseng leaves. No direct analogy was found between the birch leaf decoction and the medicinal forms of ginseng but the authors established that their effect was in the same direction in model experiments with an alteration of various organs [56].

Betulafolienetriol (7), betulafolienetriol oxide (16), 3-epiocotillol (11) and pyxinol (17) isolated from birch leaves have served as the starting materials in the synthesis of panaxoside analogs [10, 14, 57, 58]. The panaxoside 3-O-β-D-glucopyranosyl-20(S)-protopanaxadiol [10], which possesses an antitumoral action [59], has been obtained from betulafolienetriol (7) [59].

Together with triterpenoids of the dammarane series and the sesquiterpene (35), representatives of other classes of isoprenoids have been found in birch leaves. A constant component of all extracts is the principal plant sterol β-sitosterol. Another sterol — citrostadienol — has been found in the leaves of B. pendula [16]. The presence of two stereoisomeric monoterpene glucosides — 9-hydroxylinalool 9β-D-glucoside and 1-hydroxylinalool 1β-D-glucoside — has been established in the leaves of B. alba [60]. The pentacyclic triterpenoid hydroxyhopanone is present in the pollen of the male catkins and leaves of B. platyphylla var. japonica [18, 29].

TRITERPENOIDS FROM BIRCH BARK

Birch bark has two clearly distinguishable parts —outer and inner — which differ considerably in composition. The outer bark is the richest in extractive substances. Different solvents can extract from 24 to 40% on the weight of the bark. The usual methods of obtaining and analyzing the bark extracts are discussed in detail in a review by Finnish workers [7].

In extracts of the outer bark of various species of birch, pentacyclic triterpenoids of the lupane and β-amyrin series predominate. Literature information on the triterpenoids found in the outer bark is summarized in Table 2. The component of practically all extracts is betulin (41), which is responsible for the white color of birch bark [74]. The amount of betulin in the outer bark ranges from 10 to 35%, depending on the species of birch, the size and conditions of its growth, the age of the tree, the season, etc. The smallest amount of betulin had been reported for a birch with a black bark — B. dahurica [67], where this compound is a minor one, and oleanolic acid and its derivatives predominate.

The pentacyclic triterpenoids shown in Table 2 are widely distributed in nature and are present in various parts of plants of many species [75]. The only exceptions are some

esters of triterpenoids with caffeic acid — compounds (46), (47), and (58)* — and also karachic acid (57)*, which have been detected only in birch extracts.

The amount of extractive substances in the inner bark is considerably smaller, and their composition differs from that of the extracts of the outer bark. An extract of the inner bark contains a very small amount of betulin but may serve as a source of phenolic compounds [76]. The lipophilic part of an extract of the inner bark is small. The inner bark of B. verrucosa gives only 3.5% of hexane extract on the dry matter [77]. The main substances of a lipophilic extract are sterols and triterpenoids esterified with C_{14}–C_{24} fatty acids. On analysis of a saponified extract by GLC and mass spectrometry it was found that among the fatty acids linoleic predominated, while β-sitosterol and betulaprenol-7 dominated in the steroid and terpenoid groups, respectively. In the terpene group other acyclic terpenoids (sequalene, betulaprenol-6, betulaprenol-8) and cyclic terpenoids (lupeol, cycloartenol, betulin, methylenecycloartenol, citrostadienol, and monogynol-8) were also detected in minor amounts. The same sterols and terpenoids are present in a lipophilic extract of the wood [78-80].

The composition of an ethereal extract of dry B. dahurica shoots has also been studied [67]. β-Sitosterol, oleanolic acid acetate (56)*, oleanolic acid (49), and fatty acids were isolated and identified. The fraction containing the fatty acids was acetylated and was studied by the GLC method. It was established that this fraction consisted of a mixture mainly of three acids: palmitic, oleic, and linoleic.

No considerable field of application of birch bark triterpenoids has yet been found. In a review [74] devoted to betulin (41) and its use, the antiseptic properties of betulin are reported and the possibility of its use as an insecticide, an itch-relieving agent, and a component of cosmetics and shampoos is discussed. Betulin is a good emulsifying agent for fish oil—water systems, and its esters — succinate, phthalate, and tetrachlorophthalate — for the groundnut oil—water and soybean—water systems [81]. Protective coatings with improved properties have been obtained from betulin esters [82]. There is information on the physiological action of the triterpenoids of the bark. In doses on the order of 500 mg/ml, lupeol (40) and betulin (41) exhibit antitumoral activitiy (they inhibit the growth of Walker's carcinosarcoma 256) [83]. Oleanolic acid (49) and betulin (41) possess gastro- and hepatoprotective proterties [84]. Pyracrenic acid (betulinic acid caffeate) (45) exhibits antiinflammatory activity [85]. Extracts of the bark of various birch species exhibit antioxidant activity, apparently owing to the presence of triterpenoid caffeates [45].

In view of the accessibility and relative simplicity of the isolation of pentacyclic triterpenoids from the outer bark of the birch [86, 87], their use for the synthesis of terpenoids of other classes appears promising. Japanese chemists have performed a similar transformation with lupeol (40). They converted lupeol into dammarane triterpenes via baccharane, i.e., they performed a transformation that is the reverse of the biogenetic route for the synthesis of triterpenoids [88].

Betulin (41) can serve as a convenient model for developing methods for the glycosylation of triterpene alcohols [89]. Such glycosides of triterpenoids of the lupane series are also of interest by virtue of the fact that they are a component part of extracts of certain medicinal plants [90-93].

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*No compound with numbers higher than (52) are shown in Tables 1 and 2 — Translator.

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POLYSACCHARIDES OF CULTURES OF PLANT TISSUES.

I. PROPERTIES AND PARTIAL STRUCTURE

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Information is given on the structure and properties of the polysacharides (PSCs) isolated from the biomass of cultures of rose, mint, poppy, tobacco, ginseng, rose-root stonecrop, and yam using titrimetric, chromatographic, and IR and ^{13}C NMR spectroscopic methods. The monocarbohydrate compositions of the hydrolysis products have been established and methods for the practical utilization of these PSCs have been planned.

The method of obtaining biologically active compounds by cultivating plant tissues and cells is finding ever-increasing practical use. Aqueous alcoholic extracts of the biomass of cultures of ginseng and rose-root stonecrop, which possess physiological activity, are being obtained under factory conditions (Kirov, Volgograd, Kiev, etc.). A number of articles of national consumption (the "Lesnaya nimfa" ["wood nymph"] series of creams and the foaming and wetting agents "Diona" and "Iya") based on preparations from ginseng biomass have been developed and are being mass-produced.

The creation of waste-free technologies is impossible without a complex investigation of the biomass of plant cultures which, together with the desired products — glycosides, alkaloids, and essential oils— also contain other valuable substances. These are represented primarily by the carbohydrate fractions of such cultures as ginseng, rose-root stonecrop, yam, poppy, tobacco, rose, and mint. In our opinion, the wastes of these cultures can serve as a raw material for obtaining fodder proteins, nutrient substances for the microbiological industry, (after suitable treatments), etc. A comparison of the structure and properties of the polysaccharides (PSCs) of fragments of cultures of tissues and of native plants will permit a more accurate idea of the nature of carbohydrate metabolism in both cases.

We have previously described the PSCs of a whole series of essential-oil plants [1-4], and in the present communication we give the results of a study of the carbohydrate fragments of cultures of the tissues of these plants.

The PSCs of the fractions from the biomasses investigated, after the elimination of the biologically active substances and lipids, were extracted with water, oxalate buffer, and aqueous HCl solution. The PSCs were precipitated by various methods: with salt solutions, by electrolysis [5], and with alcohols. The best results were obtained in the last case

*Students T. Stenakova, A. Kirienko and L. Zubkova took part in the work.

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