EXPERIMENTAL

I.r. spectra were determined in CCl_4; NMR spectra were recorded with a Varian HA-100 spectrometer in $CDCl_3$. The spin decoupling experiments were performed by adding some C_6D_6 to the $CDCl_3$ solutions. All chemical shifts are reported in ppm (δ) relative to internal TMS. The mass spectra were measured with a Bell & Howell 21-491 mass spectrometer. The sample was inserted with a probe, and 70 e.v. ionization was used. The methyl esters obtained from the lipid moieties of the glycerides and the cyanogenetic materials were prepared by trans-esterification and characterized by GLC comparison with known standards; the GLC analyses were determined on a Varian 1520C gas chromatograph fitted with 10 ft × 0·125 in. stainless steel columns, packed with either 5% SE-30 (130°–250°, 1°/min) or 5% Carbowax 20 M (isothermal, 200°) liquid phases.

Isolation of the cyanogenetic lipids. Seeds (132 g) of *Ungnadia speciosa* (collected near the Pedernales River, on State 71, west of Austin, Texas) were ground in a Waring Blendor and extracted with *n*-pentane overnight. The pentane was removed to yield a light yellow oil (41·5 g; 31·4%). The crude oil (3·0 g) was placed on a column of silica gel (200 g) packed in hexane and eluted with hexane containing increasing amounts of ether. The cyanogenetic lipids were eluted with 2% ether in hexane. Evaporation of the solvent gave a colorless oil (0·44 g). This oil gave a strongly positive test for HCN with the picrate test.[6] The lipid materials had $[\alpha]_D^{25} = +9{\cdot}28°$ (c = 1·4, hexane) and showed a plain positive ORD curve.

The cyanogenetic lipids were chromatographed on silica gel G plates with a mixture of hexane–ether–HOAc (95:5:1). The plates were sprayed with 0·2% solution of 2′,7′-dichlorofluoroscein in ethanol and viewed in u.v. light.

Hydrogenation of the cyanogenetic lipids. The cyanogenetic lipids (0·097 g) were added to hexane containing 5% Pd-C which had been previously reduced at room temperature and atmospheric pressure. After stirring the reaction mixture for about 5 min the hydrogen uptake slowed, and the catalyst was removed by filtration. After removal of the hexane, a white solid (0·091 g, 94% yield) was obtained. The hydrogenated material had $[\alpha]_D^{25} = +27{\cdot}9°$ (c = 0·43, hexane) and showed a plain positive ORD curve.

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MONOCOTYLEDONAE

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BETA-DIKETONE, ALCOHOL AND HYDROCARBONS OF BARLEY SURFACE LIPIDS

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Abstract—Glaucous lines of barley have the β-diketone, hentriacontan-14,16-dione, in their surface lipids. The aliphatic alcohol of barley surface lipid is 1-hexacosanol, and the principal hydrocarbons have carbon numbers 25, 27, 29, 31 and 33. There is no apparent biosynthetic relationship between the primary alcohol and the β-diketone; however, there may be some relationship between the β-diketone and the hydrocarbons.

INTRODUCTION

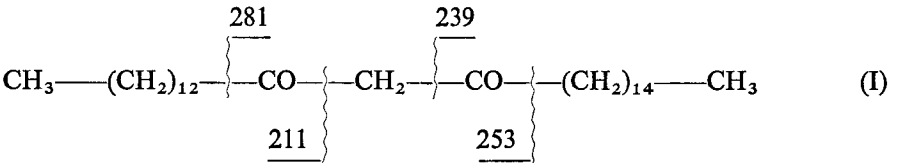
THIS study was prompted by the observation that during mid-summer 'wild type' barley had a glaucous 'bloom' and mutants of these lines were non-glaucous. Examination of the

surface lipids of the glaucous and non-glaucous lines showed that the surface lipids from the nonglaucous mutants contained little, if any, β-diketone. Long-chain β-diketones have been reported in the surface lipids of a number of plants.[1–5] The structural elucidation of the β-diketone, primary alcohol and hydrocarbons from barley surface lipids is reported here.

RESULTS AND DISCUSSION

After purification of the β-diketone by preparative TLC and complexing with copper, the GLC indicated that there was only one compound (m.p. 57°). The β-diketone accounts for 25–30 per cent of the surface lipids from glaucous barley. The i.r. spectrum had a strong band at 1610 cm^{-1} (CCl_4) and a strong u.v. maximum at 276 nm (cyclohexane). Both are characteristic of β-diketones.[2] The NMR spectrum (in CCl_4) substantiated the structure where the CH of the enolic form of the β-diketone was at 5·3 δ.

The products of alkaline hydrolysis of the β-diketone were found by GLC to be: palmitic acid, myristic acid, heptadecan-2-one and pentadecan-2-one. Oxidation of the β-diketone, followed by methylation and GLC, yielded detectable palmitic acid methyl ester and myristic acid methyl ester. The β-diketone from the surface lipids of barley is therefore hentriacontan-14,16-dione (I).



(I)

The mass spectra showed a mass peak of 464 *m/e*. Loss of water from the enol form gave a peak at 446 *m/e* (M-18). Peaks were observed at 281, 211, 239 and 253 *m/e*, displaying the fragmentation shown on the structure (I). McLafferty rearrangement[6] accounts for the peaks at 296 and 268 *m/e*. The mass spectra, then, further substantiate that the β-diketone is (I).

Hydroxy-β-diketones were observed in the surface lipid extracts and varied from slightly over one percent to undetectable in some varieties. Due to the limited quantity of hydroxy-β-diketones, only tentative identification was made by comparing the mass spectra of the isolate from barley with the hydroxy-β-diketones from wheat.[5] The mass spectra were identical, suggesting that the hydroxy-β-diketones are a mixture of 8- and 9-hydroxy-hentriacontan-14,16-diones.

In a similar study of barley surface lipids, Lundqvist *et al.*[7] observed the predominance of unidentified long chain ketones on the surface of 'wild type' barley, whereas the long chain ketones were missing almost entirely in the surface lipids from mutant strains. They also found that on plant parts where the ketones were absent primary alcohols dominated. The long chain ketones were tentatively identified as β-diketones by their complexing with

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[5] A. P. TULLOCH and R. O. WEENINK, *Can. J. Chem.* **47**, 3119 (1969).
[6] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco, Calif. (1967).
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copper and their u.v. absorption.[8] In their study they pointed out that the chemical composition of surface lipids varies markedly from one plant part to another.

Barber and Netting,[4] studying a non-glaucous mutant of wheat, found that suppression of β-diketone formation was correlated with the appearance of normal primary alcohols which were absent from the glaucous plants. They further suggested that the primary alcohols may be formed from a precursor of the β-diketones. GLC of the alcohols (as acetates) from barley surface lipids revealed that there is no difference in the alcohols from glaucous and non-glaucous barley and that there is only one alcohol with 26 carbons, which is in agreement with previous data.[9] Alcohols account for 15–20 per cent of the surface lipid extract. The i.r. spectrum indicated that the alcohol is a primary alcohol. It seems doubtful that l-hexacosanol is a precursor to the β-diketone (I) found in the surface lipids of barley.

As was observed in wheat,[4] the composition of the hydrocarbons is unchanged in the glaucous and non-glaucous mutants. The surface lipid hydrocarbons from barley heads were nearly all normal hentriacontane, whereas there were five major normal hydrocarbons with carbon numbers of 25, 27, 29, 31 and 33 on the rest of the plant (i.e. stem, leaves, sheath). Minor quantities of hydrocarbons with carbon numbers from 21 to 33 were found in both cases. Hydrocarbons compose 12–17 per cent of the surface lipid extract from heads, and 5–7 per cent of the surface lipid extract from the rest of the plant. It is noteworthy that the principal hydrocarbon of barley heads and a major hydrocarbon of the plant have the same number of carbons as the β-diketone, suggesting the possibility that they are biosynthesized via a similar pathway which branches to β-diketone in one case and hentriacontane in another. In eucalypts, suppression of β-diketone formation leads to an increase in percentage hydrocarbon with a different carbon number distribution in glaucous and non-glaucous forms.[10]

The absence of the β-diketone in the surface lipids of the non-glaucous mutants may result in more frequent further mutations. The β-diketones, nucleic acids and aromatic amino acids absorb u.v. light in about the same region of the spectrum, and the β-diketone may serve a dual role in preventing u.v. damage and desiccation in the plant. We have available a number of non-glaucous mutants, and a detailed study of their surface lipids may give more information on the role and biosynthetic pathway of the β-diketone.

EXPERIMENTAL

The plant material (*Hordeum vulgare*, Gramineae) was grown on the Montana Agricultural Experiment Station farm near Bozeman, Montana. Normal glaucous varieties: spontaneous glossy sheath mutants of the barley cultivars Barbless, Betzes, Cherron, Campana, Hanna, Piroline and Vantage; and induced mutants of Domen, Gateway and Mars were used. The mutants represent six loci (gs, gs2, gs3, gs4, gs6 and gs7) for the glossy sheath character.

Samples were taken 2 weeks after heading started, which is the time when the 'bloom' was near a maximum. The plants were cut off just above the ground and cut again just below the head. The surface lipids were extracted by dipping the plant material in hexane and holding the cut ends out of the solvent. The solvents were evaporated under vacuum or under N_2, and the lipids were stored at 4° under N_2. All solvents were analytical grade and glass redistilled prior to use.

Preparative TLC on silica gel was used to isolate the surface lipid fractions. The fractions were eluted from the silica gel with toluene, evaporated to dryness under vacuum and an aliquot was rechromatographed to test for purity. The solvent system for TLC was hexane–Et_2O–HOAc (85:15:1).

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GLC was carried out in a 0·32 × 122 cm. column packed with 1% SE-54 on Gas Chrom Z and programmed from 100 to 300° in 15 min. GLC retention times were compared to fatty acid methyl ester, hydrocarbon, and aliphatic alcohol standards from Applied Science, Inc. Other standards were synthesized in our own laboratories.

Oxidation of the β-diketone was according to the procedure of Downing and Greene,[11] except that the methyl esters were prepared using CH_2N_2.[12] Alkaline hydrolysis of the β-diketone was done by adding 2 ml of 0·5 M aqueous NaOH to 5 mg of β-diketone, and heating at 60° for 6 hr. After acidification with 1 N HCl and extraction with hexane, the methyl esters of the fatty acids were prepared using CH_2N_2.[12] Acetylation of the alcohol was done in acetic anhydride–pyridine (1:1, v/v). The i.r. spectrum of the alcohol acetate was taken using NaCl plates, and compared with the i.r. spectrum of 1-cetyl acetate and 2-cetyl acetate.

Mass spectra were determined with a Varian Mat CH-5 mass spectrometer operating at ionizing voltage 30eV, collector current 100 μA. The heated sample inlet was operated up to 200°.

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FLAVONOIDS OF *STIPA LEMMONII*

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Abstract—The presence of a number of flavonoid *O*-glycosides along with two *C*-glycosides is confirmed in *Stipa lemmonii*. The identified glycosides are: tricin 5-glucoside, 7-glucoside and 7-glucuronide; chrysoeriol 7-glucoside, 7-glucuronide and 7-rutinoside; luteolin 7-glucoside; iso-orientin and its glucoside; kaempferol and quercetin 3-acylglucosides(?).

IN THE course of a survey of certain grasses for flavonoids, a number of *O*-glycosides of tricin, chrysoeriol, luteolin, kaempferol and quercetin were found to occur along with iso-orientin and its glucoside in *Stipa lemmonii* Vasey.

Two dimensional paper chromatography of extracts of the plant revealed the presence of about fourteen flavonoids. Luteolin 7-glucoside and iso-orientin were readily identified by paper chromatography with authentic samples. The second *C*-glycoside gave glucose and a mixture of iso-orientin and orientin on acid hydrolysis, the u.v. was identical with that of iso-orientin, giving shifts with $AlCl_3$, sodium acetate and boric acid, suggesting that the glucose is attached through the glycosyl residue of iso-orientin and not through a phenolic hydroxyl group. The only other such glycoside reported is 2″-*O*-β-D-xylopyranosylvitexin.[1] No other *C*-glycosides were found in *S. lemmonii*.

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