A PHYTOCHEMICAL INVESTIGATION OF *BOTHRIOCHLOA INTERMEDIA*

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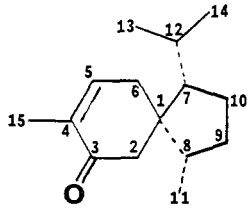
ABSTRACT.—The sesquiterpenoids acorenone-B (**1**), kessane (**2**), elemol (**3**), β-eudesmol (**4**) and 7-hydroxycalamenene (**5**) were isolated and identified in the steam volatile oil of a hybrid (56 x 627) of the grass *Bothriochloa intermedia*. In addition, five other components have been isolated but only partially characterized. The sesquiterpenes intermedeol (**6**) and neointermedeol (**7**) are the major constituents of accessions 5297 (Lahnavala, India) and 5752 (Kedah, Malaya) of *B. intermedia*, respectively. These seven well characterized sesquiterpenes were used as markers in the examination of fifteen related *Bothriochloa* grasses and all of those examined, except for two, contained either acorenone-B (**1**), intermedeol (**6**), or neointermedeol (**7**) as the major constituent. Acorenone-B (**1**) appears to reduce palatability of a grass to cows and acts as an antifeedant to grasshoppers. One of the extracts of a grass rich in acorenone-B showed limited anti-tumor activity. Details of the chemical evidence for the structure elucidation of acorenone-B are provided and complete spectral data are provided for the characterized sesquiterpenes.

Bothriochloa intermedia is a member of the Gramineae family and has been referred to by the common names sundhaur, Burnett River blue grass, poverty grass, sour grass and purple tassel grass (1). It is native in Africa, India, Pakistan, Ceylon, Assam, Burma, Malaya, Indo-China, China, Australia and most of the Pacific islands (1). Workers at the Oklahoma Experiment Station gathered seeds of *B. intermedia* from its native countries and planted and hybridized them in Oklahoma for the purpose of obtaining improved cattle grasslands (1, 2, 3). *B. intermedia* was of particular interest to the argonomists in Oklahoma because of its winter hardiness.

We, as chemists, became involved in this work when it became apparent, in the field, that the various *B. intermedia* differed markedly in palatability to cattle and some were clearly more attractive to insects than others. We thus began an investigation of the volatile oils of various *B. intermedia*. The study eventually led to a similar investigation of a few other *Bothriochloa*.

One of the grasses selected for thorough investigation by isolation procedures was a hybrid (56 x 627–1) prepared from two forms of *B. intermedia*, a gangetica type (male) indigenous to the Gangetic Plain of India, and an indica type (female) indigenous to Punjab, India. Steam distillation of the entire above ground, air dried plant material, gave 0.2% of volatile oil based on dry plant weight. Gas chromatographic analysis indicated that this oil was composed of one major component (47%), acorenone-B (**1**), whose structure was reported by us in 1968 (5), two other components in about 15% each, shown in this paper to be kessane (**2**) and elemol (**3**), respectively, and at least a dozen very minor components. We have now, in addition, identified β-eudesmol (**4**) and 7-hydroxycalamenene (**5**) in the oil and isolated five more very minor components for which the molecular formulas of four could be assigned as $C_{12}H_{22}$, $C_{15}H_{26}O$, $C_{18}H_{20}$ and $C_{15}H_{20}O_5$. We are unable, at this time, to assign a molecular formula to the fifth compound consistent with the data available.

[1]Taken in part from the Ph.D. Dissertations of James Thomas Baxter, Georgia Institute of Technology, June, 1978 and Richard James McClure, Jr., Georgia Institute of Technology, November, 1968.



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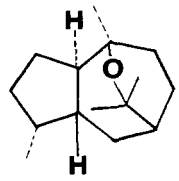

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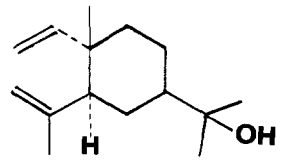

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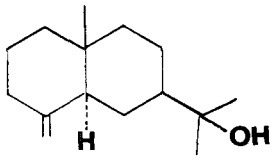

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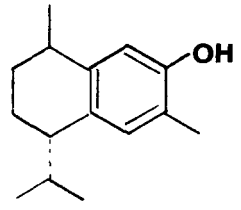

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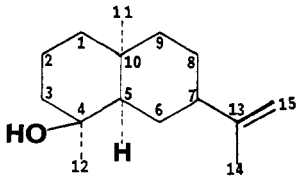

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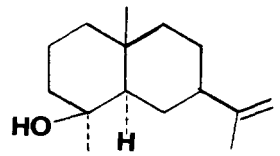

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We have previously reported the isolation of intermedeol (**6**) and neointermedeol (**7**) from *B. intermedia* accession 5297 (Lahnavala, India) and *B. intermedia* accession 5752 (Kedah, Malaya) respectively (6, 7). Intermedeol comprises about 57% of the volatile oil of accession 5297 and neointermedeol about 68% of the volatile oil of accession 5752. We then used the seven characterized sesquiterpenes, mentioned above, as markers for the different *B. intermedia* and table 1 indicates the presence, identified by isolation (I) or glc retention time (R_t) or absence of each of these sesquiterpenes in the various *B. intermedia*. In addition, there are included in table 1 several accessions of *B. glabra* and *B. insculpta* which were examined only for the presence of the three major sesquiterpenes acorenone-B (**1**), intermedeol (**6**) and neointermedeol (**7**). These three compounds had vastly different R_t; thus on a 6′ x ¼″ 5% SE 30 glass column at 152°, the R_t's were: neointermedeol (**7**) 14.4 min, intermedeol (**6**) 17.7 min and acorenone-B (**1**) 19.7 min. Every *Bothriochloa* grass listed in table 1, except for *B. insculpta* (A–5194) and *B. glabra* (A–9456) produced a single major component which was either acorenone-B (**1**), intermedeol (**6**) or neointermedeol (**7**). *B. insculpta* (A–5194)

and *B. glabra* (A–9456) contained no components previously identified by us. Those grasses which produced neointermedeol as the major constituent (access. 5752, *B. glabra* 0–675, *B. insculpta* A–9615) showed no glc detectable amounts of acorenone-B (**1**) or intermedeol (**6**). Of those plants which produced intermedeol

TABLE 1. Sesquiterpenes identified in *Bothriochloa intermedia*.[a]

Grass	Sesquiterpene							Comments
B. intermedia	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Hybrid 56 x 627–1	I	I	I	I	I	—	—	Hybrid of male gangetica form indigenous to Gangetic Plain, India and female indica form indigenous to Punjab, India.
access. 5297	—	—	—	—	—	I	—	Indigenous to Lahnavala, India.
access. 5752	—	—	—	—	—	—	I	Indigenous to Kedah, Malaya.
1–F Strain	R_t	R_t	R_t	R_t	—	R_t	—	Blend of 30 hybrids each of which contains accession 5297.
1–T Strain	R_t	R_t	R_t	R_t	—	R_t	—	Same as 1–F strain but from a different plot.
K–Strain	I	—	—	—	—	—	—	A blend of three accessions indigenous to the Kulu Valley, India.
access. 8967	R_t	—	—	—	—	R_t	—	Indigenous to Turkey.
access. 8969	R_t	—	—	—	—	R_t	—	Indigenous to Turkey.
access. 8907	—	—	—	—	—	R_t	—	Indigenous to Turkey.
B. glabra O–675	—	—	—	—	—	—	R_t	Identical by glc with *B. intermedia* access. 5752. Indigenous to Australia.
B. insculpta A–9615	—	—	—	—	—	—	R_t	Very similar to *B. intermedia* access. 5752. Indigenous to Indonesia.
B. insculpta A–5194	—	—	—	—	—	—	—	Not similar by glc to any grass examined by us. Indigenous to South Africa.
B. glabra A–9456	—	—	—	—	—	—	—	Not similar by glc to any grass examined by us. Indigenous to India.
B. glabra A–9953	—	—	—	—	—	R_t	—	Overall appearance similar to that of *B. intermedia*. access. 5297.
B. insculpta A–8587	—	—	—	—	—	R_t	—	Identical by glc with *B. intermedia* access. 5297. Indigenous to South Africa.

[a]The agronomists in Oklahoma, who supplied us with the grasses, have used the following definitions: Form—a distinct varity; Hybrid—prepared from different forms by hand emasculations and pollinations; Accession—a particular plot which could be a pure form, hybrid or strain; Strain—a blend of hybrids or accessions. Historical backgrounds of each of the grasses in Table 1 can be obtained from Dr. Robert M. Ahring (8).

(**6**) as the major constituent (access. 5297, access. 8967, *B. glabra* A-9953, *B. insculpta* A-8587) there was no glc detectable amount of neointermedeol (**7**) present and none of acorenone-B in three (access. 5297 *B. glabra* A-9953, *B. insculpta* A-8587) and only a very small peak (<5%) corresponding in R_t to acorenone-B in one other (access. 8967). Of those grasses which produced acorenone-B (**1**) as a major constituent (hybrid 56 x 627-1, 1-F strain, 1-T strain, K-strain, access. 8969), none showed any detectable amount of neointermedeol. Two (hybrid 56 x 627-1, K-strain) showed no intermedeol while three (1-F, 1-T and 8969) showed only small peaks corresponding in R_t to intermedeol. The glc curves of three of these grasses (hybrid 56 x 627-1, 1-F, 1-T) are extremely similar. Each contains large amounts of kessane (**2**) and elemol (**3**). However, in the glc trace of K-strain these peaks are insignificant and for that reason K-strain is not included in this group. K-strain is indeed unique among all of the grasses in table 1 in that its essential oil is almost pure acorenone-B (**1**)! The glc traces of the essential oils of *B. glabra* (access. 0-675), *B. insculpta* (access. A-9615) and *B. intermedia* (access. 5752) are so similar that they are indistinguishable from each other; similarly the glc traces of the oils of *B. glabra* (access. A-9953), *B. insculpta* (access. A-8587) and *B. intermedia* (access. 5297) were essentially indistinguishable from each other.

In summary, we can classify, based on glc analyses of the volatile oils, all of the *Bothriochloa* grasses listed in table 1, into three large groups, except for *B. insculpta* A-5194 and *B. glabra* A-9456 which may, therefore, not belong to this group at all. These three groups differ in whether acorenone-B (**1**), intermedeol (**6**) or neointermedeol (**7**) is the major constituent. These groups can be further divided into subgroups, in which the entire glc curve serves as a fingerprint and the grasses of the subgroup have the same fingerprint but the intensities of the peaks may vary somewhat. As mentioned above one of these subgroups is based around intermedeol (**6**) (*B. intermedia* access. 5297), one around neointermedeol (**7**) (*B. intermedia* access. 5752) and one around acorenone-B (**1**) (*B. intermedia* 1-T strain). The question may be raised as to how reproducible are the gas chromatographic analyses. In fact, we found the analyses to be amazingly reproducible. In every case, the grasses were harvested at the boot to heading stage of growth and all were grown either in Stillwater, Oklahoma or in Fort Reno, Oklahoma. We obtained hybrid 56 x 627-1, accession 5297 and accession 5752 of *B. intermedia* in several different years and the glc's of the volatile oils were consistently the same for each grass.

Only limited information is presently available regarding the palatability and insect antifeedant ability of the various grasses. However, preliminary field observations (8) suggest that large amounts of acorenone-B (**1**) in the volatile oils decrease palatability to cattle. Thus, cattle in the field found K-strain, a strain containing almost exclusively acorenone-B in its volatile oil, the least desirable of all the grasses and in a comparative situation, the cattle preferred 1-F and 1-T strains, which while still containing acorenone-B in their volatile oils, contained large amounts of elemol (**3**) and kessane (**2**), in addition to substantial amounts of monoterpenoid constituents. Similarly, grasshoppers in the field avoided plots of K-strain growing in close proximity to other grasses.

The coocurrence of acorenone-B (**1**), kessane (**2**), elemol (**3**), β-eudesmol (**4**) and hydroxycalamenene (**5**) appears to be unusual because of the diversity of

sesquiterpenoid skeleta involved. Thus, acorenone-B (**1**) and 7-hydroxycalamenene (**5**) are considered to be derived biogenetically from *trans-cis*-farnesol via the β-bisabolyl cation, whereas kessane (**2**), elemol (**3**), and β-eudesmol (**4**) are considered to be derived from *trans-trans*-farnesol via the ten-membered germacranyl cation (**9**).

We would like to record here and in the experimental section some of the chemical and physical observations on the sesquiterpene acorenone-B (**1**) not previously reported. The ^{13}C nmr spectrum of acorenone-B (**1**) confirmed the presence of an α, β-unsaturated ketone containing a single β-hydrogen and no α hydrogen by the presence of a singlet at δ 199.2, a doublet at δ 143 and a singlet at δ 135 ppm in the off-resonance decoupled spectrum. Also, the quaternary carbon at C–1 was evident from a singlet at δ 49.2 ppm. Hydrogenation of acorenone-B, in glacial acetic acid in the presence of PtO_2, gave a mixture of two dihydroacorenones-B (C–4 epimers) in a ratio of 23:77 which differed by glc retention times from the mixture (also 23:77) of dihydroacorenones obtained under similar conditions from acorenone (C–1 epimer of**1**)[2]. The epimeric mixture of dihydroacorenones-B gave a yellow crystalline 2,4-dinitrophenylhydrazone of mp 137–138°, whereas, under similar conditions, the epimeric mixture of dihydroacorenones gave no crystalline derivative. We were unable to obtain a semicarbazone derivative from the dihydroacorenones-B mixture, whereas the dihydroacorenone mixture, under similar conditions, does yield a crystalline derivative. The epimeric mixture of dihydroacorenone-B was separated, on a small scale, by preparative glc. After standing in methanol solution at room temperature for one month, each epimer partially equilibrated; complete equilibration (32:68) was obtained by refluxing in a methanolic solution of sodium methoxide. The equilibrated mixture was almost identical in composition to that obtained by hydrogenation as described above.

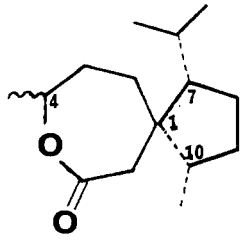
It was hoped that ord and cd could be used, especially with the two epimeric saturated dihydroacorenones-B, to give configurational and conformational information. An examination of the octant projections of the conformations of lowest energy of the two epimers of dihydroacorenone-B showed that if the cyclohexane ring adopted a chair conformation in each case with the cyclohexyl methyl group in an equatorial position one would expect a strong negative Cotton effect for one epimer and a strong positive Cotton effect in the other. In actual fact, each epimer showed a weak positive Cotton effect. This is probably the result of conformational flexibility. Finally, the absolute configuration of acorenone-B was unequivocally established by an X-ray analysis of acorenone-B 4-iodo-2-nitrophenylhydrazone (5).

Extended hydrogenation of acorenone-B in the presence of Pt/C gave a mixture of saturated 1-(2-propyl)-4,8-dimethylspiro[4.5]dec-7-ol alcohols, with one major (83%) component, which was isolated by column chromatography. Jones oxidation of this pure isomer, however, gave back the same mixture of epimeric dihydroacorenones-B as obtained directly on reduction of acorenone-B. Lithium aluminum hydride reduction of the dihydroacorenone-B mixture, obtained by hydrogenation of acorenone-B, similarly gave almost the identical mixture of saturated alcohols as obtained above. Acorenone-B was completely reduced to

[2]Kindly supplied by Dr. V. Herout, Czechoslovak Academy of Science, Institute of Organic Chemistry and Biochemistry, Prague, Czechoslovakia.

the saturated hydrocarbon 1-(2 isopropyl)-4,8-dimethylspiro[4.5]undecane as follows. The epimeric mixture of dihydroacorenones-B was converted into thioketals by the usual means. Interestingly, glc and ^1H nmr analysis indicated that a single thioketal comprised 93% of the product mixture. The thioketal was reduced with lithium in ethylamine to give one major saturated hydrocarbon, but the minor epimer could not be removed by chromatography. The ir spectrum of the isolated hydrocarbon was very similar to that recorded for acorane (10). Hydrogenation of the olefin obtained by dehydration of the saturated alcohol obtained on extended reduction of acorenone-B, mentioned above, gave this same hydrocarbon mixture.

Baeyer-Villiger oxidation of the epimeric mixture of dihydroacorenones-B with trifluoroacetic acid gave, as expected, a mixture of three lactones, with the major one comprising 73% of the mixture, which on chromatography yielded the major lactone **8** in pure form. On the basis of spectral analysis, the structure **8** could be



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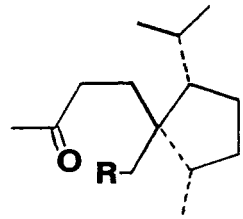
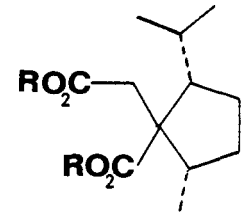


9 R=CO_2CH_3



10 R=H

11 R=CH_3

assigned. The stereochemistry at C–4 could not be determined and the absolute configurational assignments at C–1, C–7 and C–10 were based on the subsequent X-ray analysis on acorenone-B 4-iodo-2-nitrophenylhydrazone (5). Reduction of **8** gave a diol which on oxidation with chromium trioxide followed by esterification with diazomethane gave ketoester **9**. Ozonolysis of acorenone-B gave a crystalline diacid identified as **10** which was converted into its dimethyl ester **11**. The latter was found to be identical with the analogous product obtained by Sorm et al. (11) from the degradation of acorenone. This comparison established that acorenone and acorenone-B differed only in chirality at C–1. Finally, in an attempt to obtain a crystalline material suitable for X-ray analysis, a number of derivatives were prepared (see experimental) and the 4-iodo-2-nitrophenylhydrazone gave the most suitable crystals. The structure with the absolute configuration indicated in **1** was obtained from this derivative (5).

In the experimental section we have recorded spectral data previously not presented in the literature for intermedeol (**6**) (12–14) and neointermedeol (**7**) (7) for ready comparison purposes.

The ethanol extracts, their chloroform-water partitions and the hexane-aqueous methanol partitions of the chloroform partition of a number of the grasses were screened by the National Cancer Institute of the National Institutes of Health for anti-tumor activity. Only the aqueous methanol partition of K-strain showed any activity (T/C 126, 200 mg/Kg, P 388 lymphocytic leukemia tumor).

EXPERIMENTAL[3]

ISOLATION OF THE ESSENTIAL OIL OF *B. intermedia* HYBRID 56 X 627.—The air-dried, chopped grass on steam distillation gave 0.2% by weight of volatile oil after ether extraction, drying over magnesium sulfate and removal of the ether with a Vigreux column. Glc analysis (column 1, 152° and 184°) indicated the presence of a single major component, acorenone-B (**1**) (47%), two other large components, kessane (**2**) (15%) and elemol (**3**) (16%) and a dozen or more minor components.

IDENTIFICATION OF KESSANE (**2**).—Chromatography of the above volatile oil on an EM reagents silica gel 60C size C column gave kessane (**2**) in the 1:1 hexane-benzene eluent. Glc column 1 showed the kessane to be pure and mixed injection identified the peak in the original oil corresponding to kessane. Kessane was identified by comparison of its ir and ^{1}H nmr spectra with those of an authentic sample[4] (15, 16). The following data was obtained: glc column 1, R_t 10.0 min (152°), 3.6 min (184°); Calc. for $C_{15}H_{26}O$: C, 81.02; H, 11.79. Found: C, 81.34; H, 11.97; ^{13}C nmr 74.5 (s), 73.6 (s), 50.1, 41.4, 35.7, 34.7, 33.2, 32.8, 32.1, 31.0, 29.6, 28.2 (2 lines), 24.2, 18.4; *m/e* 222 (M^+, 6%), 126 (100%).

IDENTIFICATION OF ELEMOL (**3**).—The analytical sample of elemol was obtained after column and preparative gas chromatography as described above and was identical by ^{1}H nmr and ir spectra with literature reports (17, 18). It gave the following data: mp 52–53° (rpt. mp 52–53°) (17); glc column 1, R_t 10.6 min (152°); Calc. for $C_{15}H_{26}O$: C, 81.02; H, 11.79. Found: C, 81.10; H, 11.83; *m/e* 204 (M^+–H_2O, 7%), 59 (100%).[5]

IDENTIFICATION OF β-EUDESMOL (**4**).—The essential oil of *B. intermedia* hybrid 56 x 627 was distilled through an annular teflon spinning band column with a reflux ratio of 10:1. All volatile products distilling below 90° at 0.1 mm pressure were removed. Glc analysis (column 1, 152°) indicated the residue contained a major component of R_t 19.3 min and a minor component of R_t 16.7 min. The residue was chromatographed on an EM Reagents size C column and β-eudesmol (R_t 16.7 min) was eluted in the chloroform eluent. Final purification was accomplished on a Partisil M9 Whatman column. Mixed injection identified the peak in the original oil corresponding to β-eudesmol. The isolated β-eudesmol was identical by ir and ^{1}H nmr with an authentic sample.[6] The following data was obtained: mp 81° (rpt mp 82°) (19); glc column 1, R_t 16.7 min (152°), 5.5 min (184°); Calc. for $C_{15}H_{26}O$; C, 81.02; H, 11.79. Found: C, 81.05; H, 11.95; *m/e* 222 (M^+, 10%), 149 (100%).

[3]Mp's were taken on a Thomas-Hoover capillary apparatus or Thomas-Kofler micro hot stage model 651 and are uncorrected. Microanalyses were performed by Atlantic Microlabs, Atlanta, Ga. Ir spectra were recorded with a Perkin-Elmer 237B spectrometer. ^{1}H nmr spectra were obtained with a Varian T60 or JOEL-PFT-100 FT spectrometer using Me_4Si as an internal standard (δ 0); ^{13}C nmr spectra were run on the JOEL instrument. Mass spectra were obtained using either a Hitachi Perkin-Elmer Model RMU-7L or a Varian model M-66 mass spectrometer. Analytical gas chromatographic analyses were obtained either with a Hewlett-Packard Model 402 or a F&M model 400 gas chromatograph using flame ionization detectors, while preparative gas chromatography was performed on an Aerograph Autoprep Model A-700 gas chromatograph. The gas chromatography columns used with their reference numbers referred to in this paper were as follows:

Reference No.	Liquid Phase	Support	Size
1	5% SE-30	80/100 Chromosorb W	5′9″ x ¼″
2	3% OV-17	100/120 Gas-Chrom Q	5′7″ x ¼″
3	5% SE-30	80/100 Chromosorb W	4′9″ x ⅜″
4	3% OV-17	100/120 Gas-Chrom Q	4′9″ x ⅜″
5	3% Ucon Polar	80/100 Anakrom AS	6′ x ¼″

Separations were accomplished by medium pressure high performance liquid chromatography using prepacked EM Reagents, silica gel 60 size C columns and a FMI model RP-SY pump and by high pressure high performance liquid chromatography using a prepacked Partisil M9 10/25 Whatman column and a Milton Roy Model 396 Instrument minipump. Spinning band distillations were carried out using a Nester Faust annular teflon spinning band column 24 x 0.5 inches. Additional experimental details can be found in the references in footnote 1.

[4]We thank Dr. Akira Yoshikosi, The Chemical Research Institute of Non-Aqueous Solutions, Tohoku University, Sendai, Japan for ir and nmr spectra of kessane.

[5]A referee has pointed out that elemol may be an artifact of hedycaryol formed byCope-rearrangement during steam distillation or by glc analysis.

[6]We thank Professor A. R. Pinder, Department of Chemistry, Clemson University for an authentic sample of β-eudesmol.

IDENTIFICATION OF ACORENONE-B (**1**).—The steam volatile oil, mentioned above, was chromatographed on a medium pressure hplc system to yield acorenone-B in pure form in the benzene-chloroform (3:1) eluent. The isolated acorenone-B was identical in all respects with an authentic sample (5). Mixed injection identified the peak in the original oil corresponding to acorenone-B. It gave the following data: glc Column 1, R_t 19.3 min (152°), 6.3 min (184°): bp 68°/0.01 mm; n_D (30) 1.4997; d^{30}, 0.9690 g/ml; $[\alpha]^{20}D$ −13.3° (neat); ν max (film) 1670 cm^{-1}; λ max (MeOH) 242 nm (ϵ 16,300), 310 nm (ϵ 16.5); ord[7] (c, 0.006: MeOH): $[\phi]_{359}$ +368°, $[\phi]_{253}$ −4,950°, $[\phi]_{240}$ 0, $[\phi]_{220}$ +6,600°, $[\phi]_{216}$ +2,380°, a = −115; ord (C, 0.0045, hexane): $[\phi]_{359}$ +1,280°, $[\phi]_{230}$ 0, $[\phi]_{216}$ +5,450°, $[\phi]_{212}$ +2,720°, a = −96.5: cd (C, 0.0007 moles/l, MeOH): $[\theta]_{450}$ 0, $[\theta]_{322}$ +1,530°, $[\theta]_{255}$ −2,060°, Γ = 39 nm; cd (C, 0.0003 moles/l, MeOH): $[\theta]_{450}$ 0, $[\theta]_{237}$ −7,400°, $[\theta]_{218}$ 0, Γ = 23 nm; δ ($CDCl_3$): 0.77 (3 H, d, *J* 6 Hz), 0.87 (3H, d, *J* 6 Hz), 0.95 (3H, d, *J* 6 Hz), 1.75 (3H, m), 2.20 (2H, m), 2.47 (2H, AB system, *J* 17 Hz, $\Delta\nu_{AB}$ 26 Hz), 6.67 (1 H, m), irradiation at δ 2.20 caused δ 6.67 signal to collapse to a quartet (*J* 2 Hz), irradiation at δ 6.67 caused δ 1.75 signal to collapse to a triplet (*J* 2 Hz); ^{13}C nmr ($CDCl_3$): 199.2 (s), 143.5 (d), 134.7 (s), 49.2 (s), 56.7, 48.1, 45.8, 29.7, 28.9, 25.8, 25.1, 24.0, 21.1, 16.9, 15.3 ppm; *m/e* 220 (M^+, 82.8%), 135 (100%); exact mass Calc. for $C_{15}H_{24}O$ 220.183, found: 220.178 (using perfluoroalkane as reference): Calc. for $C_{15}H_{24}O$: C, 81.76, H, 10.98. Found: C, 81.53; H, 10.96%. Acorenone-B 2,4-dinitrophenylhydrazone was prepared by the usual method and gave mp 151–152° (EtOH). Calc. for $C_{21}H_{28}O_4N_4$: C, 62.98: H, 7.05. Found: C, 62.94; H, 7.39%. Acorenone-B 4-iodo-2-nitrophenylhydrazone was prepared by the addition of 38 mg of acorenone-B to a solution of 50 mg of 4-iodo-2-nitrophenylhydrazine (21) in 10 ml 95% ethanol containing one drop of conc. H_2SO_4 and heating the solution on the steam bath for 2 minutes. Chromatography on alumina gave 40 mg of the desired derivative in the benzene eluent. Mp 89–90° (benzene-95% EtOH 3:17): ν max (KBr) 3310, 1590 cm^{-1}; λ max (MeOH) 465 nm (ϵ 6,700), λ max (MeOH) 314 nm (ϵ 30,700), λ max (MeOH) 266 nm (ϵ 21,000); *m/e* 481 (M^+, 1%), 57 (100%); Calc. for $C_{21}H_{25}IN_3O_2$: C, 57.39: H, 5.87. Found: C, 52.21, H, 6.02. A crystal of this material was used for X-ray analysis (5).

IDENTIFICATION OF 7-HYDROXYCALAMENENE (**5**).—7-Hydroxycalamenene was isolated, as described for acorenone-B, from the steam volatile oil together with another compound (627–8), not yet identified, in the hexane-benzene (1:1) eluent. Pure 7-hydroxycalamenene was obtained by preparative gas chromatography (column 3, 170°). Mixed injection identified the peak in the original oil corresponding to 7-hydroxycalamenene. The isolated material was identical by ^1H nmr and ir spectra with those of authentic materials[8] (20). It gave the following data: glc column 1, R_t 9.8 min (184°); *m/e* 218 (M^+, 12%), 175 (100%); exact mass Calc. for $C_{15}H_{22}O$ 218.1670, found: 218.1685.

UNIDENTIFIED COMPOUNDS ISOLATED FROM *B. intermedia* HYBRID 56 X 627.—By a combination of spinning band fractional distillation, medium pressure hplc and preparative gas chromatography the following materials were isolated.[9]

Compound **627-A**: Glc column 1, R_t 7.3 min (152°): exact mass Calc. for $C_{12}H_{22}$ 166.172, found: 166.169.

Compound **627-3**: Glc column 1, R_t 12.4 min (152°); *m/e* 226 (M^+, 3%), 57 (100%).

Compound **627-6-B**: Glc column 1, R_t 16.7 min (152°); mp 68°; exact mass Calc. for $C_{15}H_{26}O$ 222.198, found: 222.196.

Compound **627-8**: Glc column 1, R_t 21.7 min (152°), R_t 6.9 min (184°); exact mass Calc. for $C_{15}H_{22}$ 236.157, found: 236.163.

Compound **627-10**: Glc column 1, R_t 15.9 min (184°); *m/e* 263 (98%), 149 (100%): Calc. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.42; H, 7.26%.

INTERMEDEOL (**6**).—Intermedeol was isolated from *B. intermedia* accession 5297 as previously described (6) and showed, in addition to the previously reported properties (13): ^{13}C nmr spectrum: δ ($CDCl_3$) 146.5 (s), 110.5 (t). 72.0 (s), 49.2, 43.6, 41.5, 40.5, 39.5, 35.4 (s), 23.7, 22.9, 22.5, 20.3, 18.7 and mass spectrum: *m/e* 222 (M^+, 2%), 204 (66%), 189 (52%), 161 (54%), 137 (25%), 135 (28%), 133 (31%), 123 (43%), 122 (46%), 121 (38%), 109 (46%), 107 (49%), 105 (38%), 95 (62%), 93 (58%), 91 (46%), 82 (46%), 81 (100%), 79 (52%), 77 (38%), 71 (85%), 69 (54%), 68 (34%), 67 (72%).

NEOINTERMEDEOL (**7**).—Neointermedeol was isolated from *B. intermedia* accession 5752 as previously described (7) and showed, in addition to the previously reported properties: ^1H nmr δ ($CDCl_3$) 1.02 (3 H, s), 1.12 (3 H, s), 1.72 (3H, s), 4.72 (2H, bs); ^{13}C nmr 149.6 (s), 108.1 (t), 71.7 (s), 51.8, 46.8, 44.0, 41.4 (2 peaks), 33.9 (s), 30.4, 27.0, 26.0, 20.9, 18.3 (2 peaks): mass spectrum *m/e* 222 (M^+, 6%), 206 (29%), 204 (41%), 189 (28%), 161 (22%), 137 (26%), 135 (47%),

[7]The convention used in reporting ord and cd spectra is that recommended in P. Crabbe, "Optical Rotary Dispersion and Circular Dichroism in Organic Chemistry," Holden Day, San Francisco, 1965, p. 14–20.

[8]We thank Dr. John W. Rowe, Forest Products Laboratory, USDA, Madison, Wisconsin, for these spectra.

[9]For more details on these compounds see footnote 1 J.T.B. Thesis.

123 (31%), 121 (26%), 109 (41%), 107 (29%), 105 (26%), 95 (52%), 93 (41%), 91 (27%), 82 (24%), 81 (100%), 79 (29%), 77 (22%), 71 (73%), 69 (26%), 68 (53%).

ISOLATION OF THE STEAM VOLATILE OILS—GENERAL PROCEDURES.

Procedure A. Approximately 350 g of the ground grass was placed in a 12 liter round-bottom flask which was half filled with water. After the water suspension was heated on a steam bath, steam was passed into the suspension and the steam distillate collected until there was no visual appearance of oil in the distillate. The condensate was continuously extracted with ether, the ether dried over $MgSO_4$, then evaporated with a rotary evaporator to give approximately 1 g of volatile oil.

Procedure B. Approximately 2.0 kg of ground grass was placed in a large Soxhlet extractor and continuously extracted with 9 liters of 95% ethanol for four days. Removal of the ethanol with a rotary evaporator left 162 g of green residue which was steam distilled and continuously extracted with ether for eight days. After drying, evaporation of the ether with a rotary evaporator gave 8 g of volatile oil. The oils obtained by procedures A and B were identical on glc analysis.

DIHYDROACORENONE-B (1-[2-PROPYL]-4,9-DIMETHYLSPIRO[4.5]DEC-7-ONE).—Acorenone-B (1.6 g) was hydrogenated at atmospheric pressure in glacial acetic acid in the presence of PtO_2. After the usual workup, 1.5 g of crude product was obtained, the infrared spectrum of which showed ν max (film) 3350, 1710 cm^{-1}. The crude product was reoxidized with Jones reagent to give crude dihydroacorenone (80%). Glc analysis (Column 5) showed two components in the ratio 23:77 but column chromatography (alumina and silica gel) failed to separate the mixture of epimeric ketones which showed the following properties: bp 70°/0.05 mm; $[\alpha]^{26}D$ +8.8° (C 10.2, MeOH); ν max (film) 1710 cm^{-1}; δ (CCl_4): 0.90 (6 H, d, J 6 Hz), 0.98 (3 H, d, J 6 Hz), 1.02 (3 H, d, J 6 Hz), 2.28 (2 H, AB system, J 14 Hz, $\Delta\nu_{AB}$ 27.6 Hz); m/e 222 (M^+, 13%), 41 (100%); Calc. for $C_{15}H_{26}O$: C, 81.00, H, 11.79. Found: C, 81.00, H, 12.00%.

Dihydroacorenone-B 2,4-dinitrophenylhydrazone was prepared by the usual method and gave mp 137–138° (EtOH).

COMPARISON OF DIHYDROACORENONE AND DIHYDROACORENONE-B.—A comparison of dihydroacorenone-B (mixture of epimers) with an authentic[2] sample of dihydroacorenone was made by glc (Column 5, 126°). Under these conditions dihydroacorenone-B showed two peaks with R_t 14.9 min (23%) and R_t 16.3 min (77%), while dihydroacorenone showed two peaks with R_t 13.4 min (22.5%) and R_t 15.8 min (77.5%), alone and on mixed injection.

The mass spectrum of dihydroacorenone, which showed M^+ 222 (47.6%), m/e 138 (100%), differed in the relative intensities of the various peaks as compared to dihydroacorenone-B. The infrared spectrum of dihydroacorenone differed in the 1100–1300 cm^{-1} region of the spectrum from that of dihydroacorenone-B, and its nmr spectrum differed from that of dihydroacorenone-B, particularly in the absence of a clear quartet for the $-C-CH_2-CO-$ protons.

ORD, CD AND EQUILIBRATION OF EPIMERS OF DIHYDROACORENONE-B.—The epimers of dihydroacorenone-B were separated on a small scale (1 to 2 mg) by preparative glc (Column 5). The major component (M) was obtained in 97% purity, while the minor component (m) was obtained in 85% purity, the remaining 15% being M. Component M showed the following spectral properties: λ max (CH_3OH) 283 nm (ϵ=26); ord (C, 0.288; MeOH); $[\phi]_{589}$+14.6, $[\phi]_{305}$ +830, $[\phi]_{294}$ 0, $[\phi]_{272}$−1,390, $[\phi]_{230}$−1,530° a=22.2; cd (C, 0.0105, MeOH): $[\theta]_{450}$ 0, $[\theta]_{292}$+1,510°, $[\phi]_{210}$ 0, Γ=30 nm. Both the ord and cd curves of M showed fine structure in the 300–312 nm region. Minor component 85% m showed the following spectral properties: λ max (CH_3OH) 278 nm (ϵ=22); ord (C, 0.252; MeOH): $[\phi]_{450}$+132, $[\phi]_{312}$+825, $[\phi]_{287}$ 0, $[\phi]_{274}$−167, $[\phi]_{220}$+1,050°, a=+9.9; cd (C, 0.0079; MeOH): $[\theta]_{450}$ 0, $[\theta]_{295}$+580°, $[\theta]_{210}$ 0, Γ=40 nm. The ord and cd curves of m likewise showed fine structure in the 290–320 nm region.

Partial equilibration of the above mentioned samples of 97% M and 85% m occurred on standing in methanol solution for one month; thus, at the end of this time, the samples of 97% showed 11% m and 89% M, while the sample of 85% m showed 70% m and 30% M. Equilibration of these two mixtures was obtained by refluxing methanolic sodium methoxide solutions for 6 hr; in each case the equilibrium composition was found to be 32% m and 68% M.

1-(2-PROPYL)—4,8-DIMETHYLSPIRO[4,5]DEC-7-OL.—Acorenone-B (1.2 g) was hydrogenated at atmospheric pressure and rt in glacial acetic acid in the presence of 5% Pt on C to give a crude oil (92%), the ir spectrum of which showed no C=O group but a strong hydroxyl group (ν 3300 cm^{-1}). Glc analysis showed one major component (83%) and two minor components. Chromatography on alumina gave the major component in the benzene-ether (4:1) eluent: bp 70°/0.1 mm; ν max (film) 3300, 990 cm^{-1}; δ (CCl_4): 0.82 (3 H, d, J 6 Hz), 0.90 (9 H, d, J 6 Hz), 3.07 (1H), 3.77 (1 H, m), the signal at 3.07 was reduced in intensity upon addition of D_2O; m/e 224 (M^-, 1.6%), 206 (M^+-H_2O, 40), 163 (100); Calc. for $C_{15}H_{28}O$; C, 80.29, H, 12.57. Found: 80.42, H, 12.41.

Reduction of the epimeric mixture of dihydroacorenones-B, obtained by hydrogenation of acorenone-B, with lithium aluminum hydride gave in 97% yield a mixture of three alcoholic products (74:16:10), the major component of which was shown to be identical with the major alcohol obtained on reduction of acorenone-B as mentioned above.

The major alcoholic product obtained on catalytic reduction of acorenone-B on reoxidation with Jones reagent gave the identical mixture of epimeric dihydroketones (77.5% M, 22.5% m) as obtained on hydrogenation of acorenone-B (see above).

1-(2-PROPYL)-4,8-DIMETHYLSPIRO[4.5]UNDECANE.—The epimeric mixture of dihydroacorenones-B (4.0 g) in glacial acetic acid (20 ml) was added to a solution of *p*-toluenesulfonic acid monohydrate (1 g) in 1,2-ethanedithiol (2.13 ml) and the mixture was stirred for 48 hr, then poured on crushed ice and extracted with ether. After washing with a saturated solution of sodium bicarbonate, then distilled water and drying ($MgSO_4$), evaporation gave 3.9 g of a yellow oil which was chromatographed on alumina (140 g). The petroleum ether eluent gave 3.8 g of the dithioketal which had the following properties: bp 110°/0.05 mm; δ (CCl_4) 0.81 (3 H, d, *J* 6 Hz), 0.87 (3 H, d, *J* 6 Hz), 0.97 (3 H, d, *J* 7 Hz), 1.12 (3 H, d, *J* 7 Hz), 2.10 (2 H, s), 2.50–3.00 (1 H, m), 3.17 (4 H, s); *m/e* 298 (M^-, 100%); exact mass. Calc. for $C_{17}H_{28}S_2$ 298.179, found: 298.177 (using 1,2-dichlorooctafluorocyclohexane as reference).

To a solution of the above dithioketal (2.69 g) in anhydrous ethylamine (100 g) at −20° was added 1.5 g of lithium. The dark blue solution was shaken at −20° for one hr, then water was added until the blue color disappeared. After evaporation of the ethylamine, petroleum ether was added. The solution was filtered and the filtrate dried ($MgSO_4$). The petroleum ether was removed by distillation and the residue distilled to give the saturated hydrocarbon (bp 102°/5 mm). Glc showed one predominant peak with a small shoulder; nmr showed four methyl groups and the mass spectrum showed M^- 208 (19%) and *m/e* 124 (100%). Precise mass determination by mass spectrometry using 1,2-dichlorooctafluorocyclohexane as reference gave M_{208}=208.210, $C_{15}H_{28}$ requires 208.219. Its infrared spectrum as a thick film (0.1 mm) was essentially identical to that of acorane reported by Sorm et al. (10).

1-(2-PROPYL)-4,9-DIMETHYL-8-OXASPIRO[4.6]UNDEC-7-ONE (**8**).—When dihydroacorenone-B was subjected to the Baeyer-Villiger reaction using pertrifluoroacetic acid according to the procedure of Emmons and Lucas (22), a viscous oily product (89%), the glc analysis of which showed one major (73%) and two minor (14% and 13%) products, was obtained. The ir spectrum of this crude product showed two carbonyl bands (1700 and 1725 cm^{-1}). Chromatography on alumina failed to give the pure major product, but the major product was obtained in a pure condition by crystallization from petroleum ether (bp 35–40°), or by sublimation. It showed the following data: mp 88.5–89°; ν max (KBr) 1700 cm^{-1}; λ sh (hexane) 218 nm (ϵ=98); ord (C, 0.46: hexane): $[\phi]_{359}$ 0, $[\phi]_{246}$+1,200, $[\phi]_{232}$ 0, $[\phi]_{214}$−4,150°; cd (C, 0.0194: hexane): $[\theta]_{450}$ 0, $[\theta]_{225}$ +3,030, $[\theta]_{212}$+1,800°, Γ=35 nm; δ (CCl_4) 0.87 (3 H, d, *J* 7 Hz), 1.05 (6 H, d, 6 Hz), 1.28 (3 H, d, 6 Hz), 2.58 (2 H, AB system, *J* 13 Hz, $\Delta\nu_{AB}$ 69 Hz), 4.40 (1 H, m); *m/e* 238 (M^-, 11%); 109 (100); Calc. for $C_{15}H_{26}O_2$: C, 75.52, H, 10.99. Found: C, 75.33, H, 10.81%.

PREPARATION OF KETOESTER **9**.—Lactone **8** was reduced with lithium aluminum hydride in ether to give the diol in 95% yield which exhibited the following data: glc showed a single peak; bp 90°/0.1 mm; ν max (film) 3350 cm^{-1}; δ ($CDCl_3$): 3.17 (2 H, s, OH), 3.67 (3 H, m); Calc. for $C_{15}H_{30}O_2$: C, 74.44, H, 12.50. Found: C, 74.24, H, 12.51.

The above mentioned diol was oxidized with chromium trioxide in glacial acetic acid to give a crude acidic product (53%) which was directly treated with diazomethane in ether to give crude ketoester **9** (95%). Glc analysis showed this product to contain a major component (84%) and a minor component (16%). Chromatography on alumina gave the pure major component **9** in the petroleum ether-benzene (9:1) eluent. It exhibited the following data: bp 60°/0.1 mm; ν max (film) 1720, 1730 cm^{-1}; λ max (hexane) 279 nm (ϵ 53.5), 223 nm (ϵ 156); ord (C, 0.72; hexane): $[\phi]_{589}$+15.3, $[\phi]_{304}$−52, $[\phi]_{294}$ 0, $[\phi]_{250}$−238, $[\phi]_{230}$+230°, a=−2.9; cd (C, 0.27, hexane): $[\theta]_{450}$ 0, $[\theta]_{285}$−276°, $[\theta]_{230}$ 0, Γ=50 nm; δ ($CDCl_3$): 0.82 (6 H, d, *J* 6 Hz), 0.92 (3 H, d, 6 Hz), 1.92 (3 H, s), 2.20 (2H, s, CH_2CO), 2.37 (2H, t, *J* 7 Hz, CH_2CH_2CO); *m/e* 248 (M^-, 1.4%), 131 (100); Calc. for $C_{16}H_{28}O_3$: C, 71.63, H, 10.52. Found: C, 72.00; H, 10.84.

PREPARATION OF DIACID **10** AND ITS DIMETHYL ESTER **11**.—A slow stream of ozone in oxygen (3%) was passed through a solution of acorenone-B (1.4 g) in carbon tetrachloride (25 ml) until an excess of ozone was detected in the exit gas. Water (25 ml) was added and the solution was refluxed for one hr, then steam distilled. The non-steam volatile material (0.8 g) crystallized (mp 138–140°) and was recrystallized from water to give pure **10**, which gave the following data: mp 143–145°; rpt. mp 145–147° (11); ν max (KBr) 2500–2800, 1700 cm^{-1}; δ ($CDCl_3$): 0.83–1.17 (9 H, poor resolution), 2.37 (2 H, s), 3.28 (2 H, s), 3.63 (2 H, s, OH); *m/e* 224 (M^+–H_2O, 23%), 139 (100); Calc. for $C_{15}H_{22}O_4$: C, 64.44, H, 9.15. Found: C, 64.80, H, 9.02.

Diacid **10** was converted into the corresponding dimethyl ester **11** in 91% yield with ethereal diazomethane. It showed the following properties: glc showed a single peak; bp 80°/0.05 mm; $[\alpha]_{20D}$−7.9±0.1° (C, 5.9; MeOH); ν max (film) 1730 cm^{-1}, spectrum essentially identical to that previously reported (11); δ ($CDCl_3$) 0.80 (6 H, d, *J* 6 Hz), 0.90 (3 H, d, *J* 6 Hz), 2.08 (2 H, s), 2.63 (2 H, s), 3.32 (6 H, s); *m/e* 239 (M^-–OCH_3, 16%), 153 (100); Calc. for $C_{15}H_{26}O_4$: C, 66.63, H, 9.69. Found: C, 66.69, H, 9.67.

Received 28 February, 1980.

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