Alkaloids of *Shepherdia argentea* and *Shepherdia canadensis*

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Tetrahydroharmol (**3**) was isolated from *Shepherdia argentea*. Tetrahydroharmol (**3**), serotonin (**12**), and a new alkaloid shepherdine (**2***a*) were isolated from *Shepherdia canadensis*; and evidence was obtained for the presence of 6-hydroxytryptamine in *S. canadensis*.

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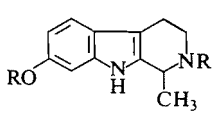
"You take the root of a cherry tree, boil it, and strain it through a cloth, and you can use it as a cure for diarrhea." So wrote Mike Oka in "A Blood Indian's Story" (1). The tree mentioned was probably buffalo or bullberry, *Shepherdia argentea* Nutt. (Eleagnaceae) (2). Our interest in this report led us to investigate the alkaloidal content of the two species of *Shepherdia* indigenous to Alberta, *S. argentea* (thorny buffalo-berry) and *S. canadensis* (Canadian buffalo-berry).

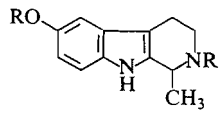
Shepherdia argentea was extracted with methanol and the crude bases isolated in the usual way; i.e., by acid–base extraction. The crude bases were acetylated, then separated by elution chromatography on deactivated alumina. Two major fractions were obtained: a mixture of non-polar acetylated bases (shown to be simple amines, *N*-acetylpyrrolidine and *N*-acetyl-*p*-anisidine were positively identified) and a crystalline compound (A).

Compound A crystallizes from ethyl acetate (m.p. 202°), is optically inactive, and has a typical indole chromophore in the ultraviolet (u.v.) (λ_{max}(EtOH) 229 (ε = 21 000), and 282 mμ (ε = 3500)) (3). The infrared (i.r.) spectrum shows indolic N—H, as well as carbonyl absorption due to an ester (1745 cm^{-1}) and an amide (1625 cm^{-1}). The nuclear magnetic resonance (n.m.r.) spectrum shows indolic N—H (τ 1.07), an ABX system characteristic of a 1,2,4-trisubstituted benzene ring, a low field methine (τ 4.35, J = 7 c.p.s.) coupled to a methyl group (τ 8.66, J = 7 c.p.s.), an ABCD system of methylene protons and two acetyl groups. The formula $C_{16}H_{18}N_2O_3$ (M^+ 286) was established by high resolution mass spectrometry. The evidence presented is consistent with either structure **1** or structure **2**.

The alkaloid itself was isolated from the crude basic extract of *Shepherdia argentea* by elution chromatography. It melts at 254.5° and the u.v. and i.r. spectra correspond closely to those reported (4) for tetrahydroharmol (**3**).



1 R = —$COCH_3$
3 R = H



2 R = —$COCH_3$
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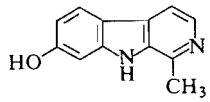

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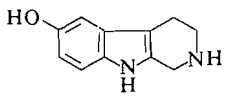
An authentic sample of **3** was prepared by reduction of harmol (**4**) (4). Acetylation gave *N*,*O*-diacetyltetrahydroharmol, identical in all respects with the compound isolated from *S. argentea*.

The basic material from *Shepherdia canadensis* was acetylated and separated by chromatography over alumina. Elution with benzene gave a compound $C_{17}H_{20}N_2O_3$, m.p. 212–214°, which is discussed below. Elution with benzene–chloroform gave a crystalline material, which had spectral properties (i.r., u.v., mass spectrometry) very similar to those of *N*,*O*-diacetyltetrahydroharmol (**1**). The n.m.r. spectrum, however, indicated that the material was a mixture (~1:1) of two very similar compounds. Fractional crystallization from methanol separated the two components. One proved to be identical with **1**, while the other, m.p. 192–194° was an isomer of **1**. Since this compound appears not to have been reported previously, we suggest the trivial name shepherdine for the unacetylated base.

N,*O*-Diacetylshepherdine crystallizes from methanol (m.p. 192–194°). The u.v. spectrum shows an indole chromophore while the i.r. spectrum shows indole N—H as well as carbonyl

absorptions due to an ester and an amide. The n.m.r. spectrum shows indole N—H, an ABX system of aromatic protons, a low field methine coupled to a methyl group, an ABCD system of methylene protons, and two acetyl groups. The molecular formula $C_{16}H_{18}N_2O_3$ (M^+ 286) was established by high resolution mass spectrometry. The evidence presented is consistent with either strucutre **1** or **2**, but since it has been shown (see above) that *N,O*-diacetylshepherdine is different from **1**, structure **2** is indicated for *N,O*-diacetylshepherdine. Recently, another harmala-type alkaloid, plectocomine (**5**), which has an oxygen function at C-6, has been identified (5).



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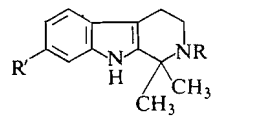
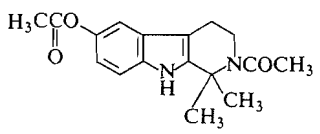
Sulfuric acid catalyzed condensation of 5-benzyloxytryptamine with acetaldehyde (6), followed by acetylation, hydrogenolysis (Pd–C), and acetylation gave *N,O*-diacetylshepherdine (**2**). The melting point (192–194°) and i.r. spectrum were identical with those of the acetylated alkaloid. Shepherdine thus has structure **2***a*.

The compound (m.p. 212–214°) referred to above also shows an indole chromophore in the u.v. The i.r. spectrum shows indole N—H as well as carbonyl absorption due to an ester and an amide. The n.m.r. spectrum shows indolic N—H, an ABX system of aromatic protons, an AA′BB′ system of methylene protons, two acetyl groups, and a *gem*-dimethyl group (τ 8.16). The molecular formula $C_{17}H_{20}N_2O_3$ (M^+ 300) was established by high resolution mass spectrometry. The evidence presented is consistent with either structure **6** or **7**.



6 R = $COCH_3$; R′ = $OCOCH_3$
8 R = H; R′ = H
9 R = $COCH_3$; R′ = H



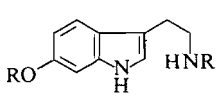
7

Since the signal in the n.m.r. assigned to the *gem*-dimethyl group occurs at lower field than expected, a model compound was prepared to determine whether *N*-acetylation markedly deshields the *gem*-dimethyl group in this situation. *p*-Toluene sulfonic acid catalyzed condensation of tryptamine with acetone to form the Schiff base, followed by phosphorus oxychloride catalyzed ring closure and then hydrolysis yielded compound **8** (7). The n.m.r. spectrum of compound **8** shows a six-proton singlet due to the *gem*-dimethyl group at τ 8.54. Acetylation of compound **8** gives compound **9**, the n.m.r. of which shows the *gem*-dimethyl group at τ 8.09, confirming that *N*-acetylation does cause considerable deshielding.

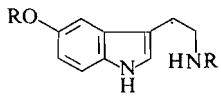
The hypothesis that indole alkaloids are derived in part from tryptophan is generally accepted (8*a*, *b*). Thus an alkaloid of structure **6** or **7** could be derived either from a suitably oxygenated tryptophan and acetone, or by methylation of a suitable tetrahydroharmol precursor. Acetone or its biological equivalent is not a common biosynthetic building block and we suspected that the compound might be an artifact formed during the isolation. Tryptamines with a C-6 oxygen substituent have been shown to condense readily with acetone under mild conditions to form ring closed condensation products (7). To determine whether or not the acetylated compound was an artifact, *S. canadensis* was extracted with acetone-free methanol. Examination (chromatography, mass spectrometry) of the crude basic extract after acetylation showed no trace of the *gem*-dimethyl compound. Structure **6** is thus suggested on this basis. The precursor which gives rise to **6**, assumed to be 6-hydroxytryptamine, was not isolated.

The last acetylated alkaloid characterized shows an indole chromophore in the u.v. as well as indolic N—H and ester and amide carbonyl in the i.r. The n.m.r. spectrum shows indolic N—H, an ABX system of aromatic protons, an aromatic proton alpha to the indole N—H, an AA′BB′ system of methylene protons, an amide N—H (τ 6.77), and two acetyl groups. The molecular formula $C_{14}H_{16}N_2O_3$ (M^+ 260) was established by high resolution mass spectrometry. The evidence presented is consistent with either structure **10** or **11**.

Serotonin creatinine sulfate was acetylated and creatinine was removed. *N,O*-Diacetylserotonin



10 R = $COCH_3$



11 R = $COCH_3$
12 R = H

thus formed has an i.r. spectrum superimposable on the i.r. spectrum of the acetylated naturally occurring compound. Thus the acetylated alkaloid has structure **11**. It is assumed that this is present in the plant as serotonin (**12**).

It is interesting to note that spin–spin decoupling experiments with **11** reveal a small coupling between indolic N—H and H-4. To the best of our knowledge, this type of coupling has not previously been reported for indoles.

Both the harmala alkaloids and tryptamines show psychotomimetic activity (*9a*, *b*). Whether this property stimulated the use of these plants in folk medicine is a matter for conjecture. It is also of interest to note that various synthetic ethers of harmol find use in treatment of amebic dysentery (10).

Experimental

Optical rotatory dispersion (o.r.d.) spectra were measured in methanol using a Jasco Optical Rotatory Dispersion Recorder model ORD/UV-5.

The u.v. spectra were measured in 95% ethanol, unless otherwise specified, using a Perkin–Elmer Ultraviolet Spectrophotometer model 202 or a Jasco Optical Rotatory Dispersion Recorder model ORD/UV-5.

The i.r. spectra were measured in chloroform solution, unless otherwise specified, using a Perkin–Elmer model 337 grating i.r. spectrophotometer.

The n.m.r. spectra were measured in deuteriochloroform, unless otherwise specified, using a Varian Associates model HR-100 spectrophotometer with tetramethylsilane as internal standard.

Mass spectra were recorded on an A.E.I. model MS-9 mass spectrometer.

Melting points were determined on a hot-stage Leitz melting point apparatus or a hot-stage Fisher–Johns melting point apparatus and are uncorrected.

Plant Materials

Shepherdia argentea was collected from the Lethbridge area by A. Johnston and from the University of Alberta Experimental Farm by R. H. Knowles.

Shepherdia canadensis was collected near the North Saskatchewan river in the Edmonton area and was identified by R. G. H. Cormack, Department of Botany, University of Alberta.

Extraction and Isolation of the Crude Bases of Shepherdia argentea

The bark of the roots (880 g) of *Shepherdia argentea* was ground, air dried, and extracted with 5% aqueous methanol in a Soxhlet extractor. Excess methanol was removed *in vacuo*, water added, then traces of methanol removed *in vacuo*. Five percent aqueous hydrochloric acid was added until the p*H* was less than 5 and the insoluble material filtered and discarded. The acidic and neutral components were removed from the filtrate by ether extraction. The acidic solution was basified with aqueous ammonia to p*H* 10 and the resultant precipitate filtered. The basic filtrate was continuously extracted with ether for 48 h. The ether extract was dried (anhydrous magnesium sulfate) and excess solvent removed *in vacuo* to give 1.7 g crude bases (0.2%). Further purification of the crude alkaloidal material was achieved by a second acid–base extraction.

Acetylations

The base(s) were dissolved in one part pyridine, two parts acetic anhydride added, and the reaction mixture allowed to stand at room temperature at least 12 h. Excess solvents were removed by azeotropic distillation with toluene *in vacuo*.

Isolation of Acetylated Bases of Shepherdia argentea

Crude acetylated bases were separated by elution chromatography on Woelm alumina, activity 3. Two fractions were obtained: (*1*) non-polar bases, eluted with benzene–chloroform, 10:1 (36%); (*2*) *N,O*-diacetyltetrahydroharmol, eluted with benzene–chloroform, 5:1 (24%).

N,O-Diacetyltetrahydroharmol (1)

Compound **1** was isolated by elution chromatography on deactivated alumina with benzene–chloroform (5:1). Compound **1** crystallizes from ethyl acetate, m.p. 202°. The u.v. spectrum: λ_{max} 229 (ε = 21 000) and 282 mμ (ε = 3500). The i.r. spectrum: ν_{max} 3450, 3300, 1745, 1625 cm^{-1}. The n.m.r. spectrum: τ 1.07, singlet (N—*H*); 2.72, doublet, *J* = 8 c.p.s. (H-5); 3.01, doublet *J* = 2 c.p.s. (H-8); 3.26, doublet of doublets, *J* = 2, 8 c.p.s. (H-6); 4.35, quartet, *J* = 7 c.p.s. (H-1); 6.08, multiplet (H-3e); 6.65, multiplet (H-3a); 7.34, multiplet (H-4e,4a); 7.73, singlet (N—CO—CH_3); 7.82, singlet (O—CO—CH_3); 8.66, doublet, *J* = 7 c.p.s. (C_1—CH_3). Assignments have been verified by spin–spin decoupling experiments. Mass spectrum: *m/e* 286 ($C_{16}H_{18}N_2O_3$; found 286.1317, calculated 286.1317), 271, 244, 229 (base), 201, 187. The o.r.d. spectrum: no rotation.

Tetrahydroharmol (3)

Compound **3** was isolated by elution chromatography of the crude bases of *S. argentea* on deactivated alumina using chloroform–methanol (20:1). Compound **3** thus obtained melted at 254–255°; reported 256° (4). The u.v. spectrum: λ_{max} 229 (log ε = 4.57), 270 (log ε = 3.77), and 299 mμ (log ε = 3.85). The i.r. spectrum: ν_{max}(Nujol) 3380, 3265, 3245, 1620, 1560 cm^{-1}. Mass spectrum: *m/e* 202 ($C_{12}H_{14}N_2O$; found 202.1104, calculated 202.1106), 187 (base), 172, 159.

Tetrahydroharmol was dissolved in a minimum amount of hot methanol and methanolic HCl added until the solution was acidic. Excess solvent was removed. Tetrahydroharmol hydrochloride crystallizes from methanol–ether, m.p. 230° (dec.), reported 235° (4). Several months later, the same sample of tetrahydroharmol hydrochloride melted at 235° without decomposition.

Isolation of Non-polar Bases of Shepherdia argentea by Gas Chromatography

The non-polar acetylated bases separated by gas chromatography (g.c.) (Aerograph Manual Temperature Programmed Gas Chromatograph model A-90-P3, equipped with a thermal conductivity detector, 10′ × 1/4″, 5% SE-30 column, column temperature 205°, flow rate (He) 20 ml/min) are shown in Table 1. Apparent molecular formulas as determined by mass spectrometry are given in parentheses.

The compounds with retention time of 6.5 and 23.3 min were identified as *N*-acetylpyrrolidine and *N*-acetyl-*p*-anisidine, respectively, by comparison (g.c., mass spectra) with authentic samples.

Extraction and Isolation of Crude Bases of Shepherdia canadensis

The bark of roots (610 g) was extracted by the procedure reported for *S. argentea*, yielding 2.2 g crude bases (0.36%). The crude bases were acetylated in the usual manner and chromatographed over Woelm alumina, activity 3. Four major fractions were isolated, as shown in Table 2.

7-Acetoxy-2-acetyl-1,1-dimethyl-1,2,3,4-tetrahydro-2-carboline (6)

Compound **6** was isolated by elution chromatography over deactivated alumina using benzene. Compound **6** crystallizes from methanol, m.p. 212–214°. The u.v. spectrum: λ_{max} 227, (ε = 10 300) and 291 mμ, (ε = 2000). The i.r. spectrum: ν_{max} 3455, 3300, 1750, 1640 cm^{-1}. The n.m.r. spectrum: τ 1.58, singlet (N—*H*); 2.60, doublet, *J* = 8 c.p.s. (H-5); 2.94, doublet, *J* = 2 c.p.s. (H-8); 3.20, doublet of doublets, *J* = 2, 8 c.p.s. (H-6); 6.36, triplet (H-3e,3a); 7.22, triplet (H-4e,4a); 7.68, singlet (N—CO—CH_3); 7.75, singlet (OCOCH_3); 8.16, singlet (C(CH_3)$_2$).

TABLE 1

Gas chromatogram of acetylated non-polar bases

Retention time (min)	Molecular ion (*m/e*)
6.5	113 ($C_6H_{11}NO$)
8.8	157 ($C_8H_{15}NO_2$)
9.5	—
10.6	—
16.0	163 ($C_{10}H_{13}NO$)
23.3	165 ($C_9H_{11}NO_2$)

TABLE 2

Chromatogram of acetylated bases

Fraction	Eluent	Contents
1	Benzene	Simple acetylated bases
2	Benzene	Artifact of extraction
3	Benzene–chloroform (5:1)	*N,O*-Diacetyltetrahydroharmol (35%); *N,O*-diacetylshepherdine
4	Chloroform–methanol (100:1)	*N,O*-Diacetylserotonin (17%)

Assignments have been verified by spin–spin decoupling experiments. Mass spectrum: *m/e* 300 ($C_{17}H_{20}N_2O_3$; measured 300.1475, calculated 300.1474), 285, 258, 243 (base), 201, 200.

Synthesis of 2-Acetyl-1,1-dimethyl-1,2,3,4-tetrahydro-2-carboline (9) (7)

Tryptamine (0.9 g), acetone (3.5 ml), and *p*-toluenesulfonic acid (catalytic amount) in anhydrous benzene (30 ml) were refluxed under nitrogen for 3.5 h with azeotropic distillation of water. The solution was cooled, filtered over anhydrous potassium carbonate, and excess solvent removed *in vacuo*. The residue was suspended in anhydrous benzene and freshly distilled phosphorus oxychloride (2.5 ml) added. The mixture was allowed to stand at room temperature 1 h, then refluxed 1 h. Excess solvent was removed *in vacuo* under nitrogen. The residue was heated briefly with water (30 ml), filtered, decolorized with Norit, basified (p*H* 9) with ammonia, then extracted with ether. The ether fraction was dried (anhydrous magnesium sulfate) and excess solvent removed *in vacuo* to give 1,1-dimethyl-1,2,3,4-tetrahydro-2-carboline (**8**). Compound **8** was purified by elution chromatography on Woelm alumina, activity 3. Compound **8** crystallizes from benzene, m.p. 94–96°, reported 111.5–115.5° (7). The n.m.r. spectrum: τ 2.20 singlet (N—*H*); 2.90, multiplet (4 Ar—*H*); 6.80, triplet (H-3e,3a); 7.30, triplet (H-4e,4a); 8.00, singlet (N—*H*); 8.54, singlet (C—(CH_3)$_2$).

Compound **8** was acetylated in the usual manner giving **9** which crystallizes from acetone–water, m.p. 228–229°. The n.m.r. spectrum: τ 1.67, singlet (N—*H*); 2.72, multiplet (4 Ar—*H*); 6.30, triplet (H-3e,3a); 7.16, triplet (H-4e,4a); 7.70, singlet (NCOCH_3); 8.09, singlet (C—(CH_3)$_2$).

Extraction and Isolation of Bases of Shepherdia canadensis Using Reagent Methanol

Dried, ground roots (710 g) of *S. canadensis* were extracted with reagent methanol (free from acetone) in a Soxhlet extractor. Excess solvent was removed *in vacuo*, water added, and traces of methanol removed. The aqueous extract was acidified (p*H* 3) with 5% aqueous hydrochloric acid, then extracted with ether to remove neutral and acidic material. The aqueous acidic fraction was basified (p*H* 9) with sodium carbonate, the precipitate filtered, and the filtrate continuously extracted with ether for 48 h. The ether fraction was dried (anhydrous magnesium sulfate) and excess solvent removed *in vacuo* yielding crude bases (3.3 g). The crude bases (1.3 g) isolated by extraction of *S. canadensis* with reagent methanol were acetylated in the usual manner yielding crude acetylated bases (2.1 g). Mass spectrum: *m/e* 286, 260, 229, 175. The mass spectrum of the crude acetylated bases obtained using technical grade methanol showed an additional peak at *m/e* 300. Elution chromatography of the crude acetylated bases on Woelm alumina, activity 3, showed no trace of compound **6**.

Isolation of N,O-Diacetyltetrahydroharmol from Shepherdia canadensis

N,O-diacetyltetrahydroharmol was isolated by elution chromatography of the crude acetylated bases of *S. canadensis* on deactivated alumina using benzene–chloro-

form (5:1). It was separated from *N,O*-diacetylshepherdine by fractional crystallization from methanol. It has a melting point 202° and its spectral properties are identical with those reported for compound **1**.

Synthesis of N,O-Diacetyltetrahydroharmol (1) (4)

Sodium (1.5 g) was added to a refluxing solution of harmol (0.1 g) (Aldrich Chemical Co.) in anhydrous ethanol under a nitrogen atmosphere. When all the sodium was dissolved, the solution was acidified (aqueous HCl), the precipitate filtered and washed with ethanol. The ethanol washings and filtrate were combined and excess solvent removed *in vacuo*. The crude residue was dissolved in water, the solution basified with aqueous ammonia, then extracted with ether. The ether was dried (anhydrous magnesium sulfate), then removed *in vacuo* to give tetrahydroharmol (**3**). Tetrahydroharmol was acetylated in the usual manner and the crude acetate purified by elution chromatography over Woelm alumina, activity 3. *N,O*-Diacetyltetrahydroharmol crystallizes from ethyl acetate, m.p. 202°. Its spectral properties are identical with those of the naturally occurring alkaloid.

N,O-Diacetylshepherdine (2)

N,O-diacetylshepherdine was isolated by elution chromatography of the crude acetylated bases of *S. canadensis* on deactivated alumina using benzene–chloroform (5:1). It was separated from *N,O*-diacetyltetrahydroharmol by fractional crystallization from methanol. It crystallizes from methanol, m.p. 192–194° and gives a depressed mixed melting point with *N,O*-diacetyltetrahydroharmol, m.p. 165–175°. The u.v. spectrum: λ_{max} 226 (ε = 31 400) and 281 mμ (ε = 6300). The i.r. spectrum: ν_{max} 3450, 3275, 1745, 1630 cm^{-1}. The n.m.r. spectrum: τ 1.14, singlet (N—*H*); 2.80, doublet, J = 8 c.p.s. (H-8); 2.92, doublet, J = 1 c.p.s. (H-5); 3.22, doublet of doublets, J = 1, 8 c.p.s. (H-7); 4.30, quartet, J = 6 c.p.s. (H-1); 6.07, multiplet (H-3e); 6.64, multiplet (H-3a); 7.34, multiplet (H-4e,4a); 7.71, singlet (NCOC*H*$_3$); 7.80, singlet (OCOC*H*$_3$); 8.62, doublet, J = 6 c.p.s. (C—C*H*$_3$). Assignments have been verified by spin–spin decoupling experiments. Mass spectrum: 286 (base) ($C_{16}H_{18}N_2O_3$; found 286.1317, calculated 286.1317), 271, 244, 229, 201, 187.

Synthesis of N,O-Diacetylshepherdine (2) (6)

5-Benzyloxytryptamine (0.1 g) (Aldrich Chemical Co.), 2 *N* sulfuric acid (5 drops), dioxane (1 ml), and water (1 ml) were cooled under nitrogen and freshly prepared 10% aqueous acetaldehyde (2 ml) added. The mixture was allowed to stand at room temperature. After 0.5 h, a crystalline precipitate formed which was collected and acetylated in the usual manner to give 2-acetyl-6-benzyloxy-1-methyl-1,2,3,4-tetrahydro-2-carboline. It crystallizes from acetone–methanol, m.p. 179.5–181°. The crystalline compound (0.4 g) and 30% palladium–charcoal (catalytic amount) in reagent methanol (15 ml) were hydrogenated at room temperature and atmospheric pressure for 4 h, i.e., until starting material had all reacted (t.l.c.). The solution was filtered, excess solvent removed *in vacuo* and the crude residue acetylated in the usual way. The crude acetyl derivative was purified by elution chromatography on Woelm alumina, activity 3, to give **2** which crystallizes from methanol, m.p. 192–194°. Its melting point, mixed melting point, and i.r. spectrum are identical with those of the naturally occurring *N,O*-diacetylshepherdine.

N,O-Diacetylserotonin (11)

N,O-Diacetylserotonin was isolated by elution chromatography of the crude acetylated bases of *S. canadensis* over deactivated alumina using chloroform. *N,O*-Diacetylserotonin could not be crystallized. The u.v. spectrum: λ_{max} 224 (ε = 27 000) and 287 mμ (ε = 5500). The i.r. spectrum: ν_{max} 3500, 3325, 1750, 1675 cm^{-1}. The n.m.r. spectrum: τ acetone-d_6 −0.20, singlet (N—*H*); 2.68, doublet, J = 8 c.p.s. (H-7); 2.75, doublet, J = 2 c.p.s. (H-4); 2.86, singlet (H-1); 3.21, doublet of doublets, J = 2, 8 c.p.s. (H-6); 6.58, multiplet (H-2′e,2′a); 6.77, broad singlet (N*H*COCH$_3$); 7.15, triplet (H-1′e,1′a); 7.79, singlet (NCOC*H*$_3$); 8.15, singlet (OCOC*H*$_3$). Assignments made have been verified by spin–spin decoupling experiments. In addition, irradiation at 1020 c.p.s. causes sharpening of signals at τ 2.75 and 2.86 indicating a small W coupling between the indole N—H and H-4, and a small coupling between indole N—H and H-1. Irradiation at 716 c.p.s. causes sharpening of the signal at τ 3.21 showing a small para coupling between H-4 and H-7. Mass spectrum: *m/e* 260 ($C_{14}H_{16}N_2O_3$; found 260.1158, calculated 260.1161), 201, 188, 159, 146.

Synthesis of N,O-Diacetylserotonin (11)

Serotonin creatinine sulfate (Aldrich Chemical Co.) (9.1 g) was acetylated in the usual manner. Creatinine was removed by addition of acetone and filtering the precipitate. Excess acetone was removed *in vacuo* and crude **11** purified by elution chromatography on Woelm alumina. *N,O*-Diacetylserotonin has an i.r. spectrum identical with that of the naturally occurring compound.

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