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# THE SYNTHESIS OF *CYPRIDINA* ETIOLUCIFERAMINE AND THE PROOF OF STRUCTURE OF *CYPRIDINA* LUCIFERIN[a]

T. P. KARPETSKY* and E. H. WHITE†
Department of Chemistry The Johns Hopkins University Baltimore, Maryland 21218

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**Abstract**—An unambiguous synthesis of *Cypridina* etioluciferamine was accomplished in order to prove the structure of this important bioluminescent natural product. Several 2-aminopyrazine 1-oxides were synthesized in order to establish a spectroscopic method for determining the placement of substituents on the pyrazine nucleus of *Cypridina* etioluciferamine. Titanium tetrachloride was used to improve the yields of these compounds; for example, the yield of 2-amino-3-methyl-5-phenylpyrazine 1-oxide (**19**) from reaction of phenylglyoxal 1-oxime and α-aminopropionitrile was raised from 3% to 51% by the use of titanium tetrachloride. The pyrazine ring proton is found at τ 1·37 (DMSO-$d_6$). The isomeric 2-amino-3-methyl-6-phenylpyrazine 1-oxide (**22**) was similarly prepared and its pyrazine ring proton is found at τ 2·18. This large difference (0·81 ppm) in chemical shift was used to determine whether a 2-aminopyrazine 1-oxide was 5- or 6- substituted. Prepared in an analogous fashion were 2-amino-5-(indol-3-yl)-3-methylpyrazine 1-oxide (**23**) and 2-amino-5-(indol-3-yl)-3-(3-phthalimidopropyl)pyrazine 1-oxide (**16**). The structures of these compounds were verified by NMR spectroscopy. By treatment with Raney nickel and hydrogen gas, then 100% hydrazine hydrate, **16** was converted to 2-amino-3-(3-aminopropyl)-5-indol-3-ylpyrazine (**5**), isolated as the dihydrochloride. This compound, with the indole moiety definitely placed at C-5, is identical with *Cypridina* etioluciferamine dihydrochloride (IR, UV, TLC). These results show that the structures of *Cypridina* etioluciferamine and luciferin are correct as published.

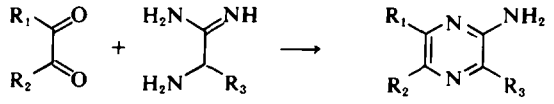
The bioluminescent reaction of *Cypridina* luciferin and luciferase was first noted by Harvey in 1917.[2] The reaction is simple, requiring only substrate (luciferin), enzyme (luciferase) and oxygen for light production.[3] Therefore, the *Cypridina* system is ideal for chemical and kinetic studies aimed at elucidating the mechanism of bioluminescence.[4] A necessary prequisite for these studies, however, is an unambigous chemical structure for *Cypridina* luciferin.

Using impure materials, various groups have proposed partial structures for the luciferin.[5-10] In 1957, Shimomura, *et al.* crystallized *Cypridina* luciferin;[11] this group proposed a structure that was later shown to be incorrect.[12] Investigations of the properties of luciferin showed that, in the presence of oxygen and luciferase,[13] it is converted to two compounds termed oxyluciferin and etioluciferin with concomitant emission of light.[14-16] Oxyluciferin is the initial product of the light reaction; treatment of this compound with dilute hydrochloric acid affords etioluciferin. Since etioluciferin is more stable to oxygen, acids, and alkalis than its precursors, attention was then focused on the structure of this degradation product. On the basis of spectral and degradative evidence, Goto, *et al.* proposed structure **1** for *Cypridina* etioluciferin. Their evidence for choosing structure **1** instead of **2** is not compelling. The mass spectrum of etioluciferamine[17] (**1** or **2**, $R_3 = (CH_2)_3NH_2$, obtained by hydrolysis of etioluciferin) can be rationalized as corresponding to either isomer **1** or **2**. These workers also based their choice on the amino acid analysis of luciferin,[17] although pathways can be written for the decomposition of the luciferin structure derived from **2** which would give the same amino acid analysis. A total synthesis of *Cypridina* luciferin did not clear up this ambiguity in the structure of etioluciferin.[18] The condensation reaction shown in Eq 1 was used to prepare the key intermediate **3** (**1** with $R_3 = (CH_2)_3NHCOPh$). It is possible that this reaction could give **4** (**2** with $R_3 = (CH_2)_3NHCOPh$) but the authors claimed to isolate only **3** from the reaction mixture. They gave no support for this assignment at the time but whichever isomer they actually had was converted to authentic *Cypridina* luciferin.

In papers published after the structure proof and synthesis of *Cypridina* luciferin, Goto, *et al.* explored the possibility that the reaction shown in Eq 1 would give isomers corresponding to **2** or **4** rather than **1** or **3**.[19,20] This work was not conclusive because in no case was a *pair* of isomers isolated and examined for differences in physical properties. Certain NMR evidence cited by Goto for the posi-

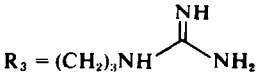
[a] A preliminary account of this research has been published.[1]

†To whom requests for reprints should be sent.

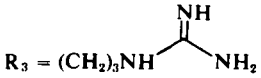


(1)

1: $R_1$ = H, $R_2$ = indol-3-yl



2: $R_2$ = H, $R_1$ = indol-3-yl



5: $R_1$ = H, $R_2$ = indol-3-yl
$R_3 = (CH_2)_3NH_2$

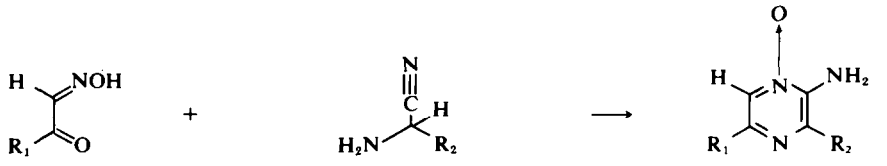
tion of substituents on 2-aminopyrazine rings is also greatly weakened by the fact that isomeric compounds were not prepared and studied.[19] The bulk of these studies were carried out with phenyl-substituted pyrazines. It is also possible that indole glyoxals might give rise to pyrazine products whose ring substitution patterns differ from those pyrazines obtained from phenylglyoxals. Therefore, since the condensation shown in Eq 1 with indoleglyoxal as reactant may be a special case leading only to **4** rather than **3**, and since the physical properties of such isomers as **2** are unknown, the assigning of structure **1** as that of *Cypridina* etioluciferin is premature. Although the positioning of the indole moiety on the pyrazine nucleus of *Cypridina* luciferin and its degradation products is questioned, the remainder of the structure appears secure.

A mechanism has been postulated for the bioluminescent reaction which fits the observed chemical transformation of *Cypridina* luciferin to oxyluciferin.[21,22] Recently, a high yield synthesis of racemic luciferin from etioluciferin has appeared;[23] this has shifted the difficulty in synthesizing luciferin towards the preparation of etioluciferamine. We therefore felt that by *unambigously* synthesizing **5** (**1** with $R_3 = -(CH_2)_3-NH_2$), the compound purported to be *Cypridina* etioluciferamine, we could accomplish several important ends at the same time. The question of the exact structure of etioluciferamine would be settled. Also, through the use of model compounds, spectroscopic means would be found to differentiate isomeric pyrazines related to **1** and **2**. Finally, we wanted to achieve a shorter synthesis of **5** than that previously published.

The method of Sharp and Spring for the synthesis of pyrazine rings[24,25] was ideally suited to our synthetic aims. Only one 2-aminopyrazine 1-oxide is isolated and the substituents are on the 3- and 5-positions, exactly as required for the preparation of **5**. In addition, the 2-amino and indole ($R_2$) moieties can be introduced directly rather than constructed at a later state. The use of this reaction in our synthetic scheme immediately defined two compounds to be synthesized: indol-3-ylglyoxal 1-oxime (**6**) and 2,5-diamino-*n*-valeronitrile, the latter having the 5-amino group protected in some manner (**7**).

## RESULTS

*Indol-3-ylglyoxal 1-oxime.* The synthesis of **6** began with the preparation of ethoxycarbonylacetyl chloride(**8**) from potassium ethyl malonate and thionyl chloride.[26-28] The preparation of **9** by the route shown in Eq 3 was accomplished previously by Baker.[29,30] The solvent of choice for acylating indole *via* indolymagnesium bromide[29-32] or chloride is anhydrous diethyl ether.[33] It has been reported that for the acylation of indolylmagnesium bromide by ethyl chloroformate, the higher the reaction temperature, the greater the ratio of 3-substituted to 1-substituted indole product obtained.[34] This effect of temperature on yield was ob-

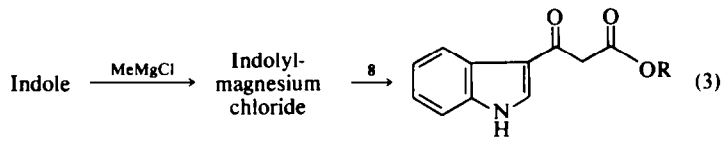


(2)

6: $R_1$ = indol-3-yl
17: $R_1$ = phenyl

7: $R_2 = (CH_2)_3$-N (protecting groups)
18: $R_2$ = Me

19: $R_1$ = phenyl; $R_2$ = Me
23: $R_1$ = indol-3-yl; $R_2$ = Me




**9**: R = Et
**11**: R = H

served by us in the preparation of **9**, the yield of **9** increasing from 9% at −60° reaction temperature, to 13% at −7° and 24% at +35°.

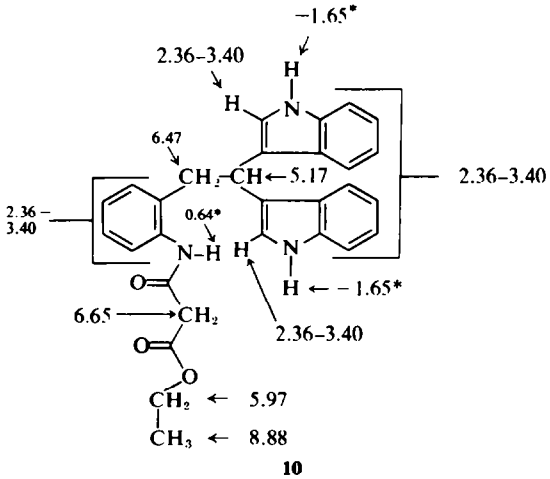
The IR spectrum of **9** (KBr) displays the characteristics of a 3-acylindole system, *i.e.*, both a low-frequency N–H stretch (3215 $cm^{-1}$ compared with 3490 $cm^{-1}$ for indole[35]) and a low-frequency CO stretch (1615 $cm^{-1}$). This lowering of frequency of both N–H and β-indole C=O stretch is believed to be due to extremely strong intermolecular H—bonding,[36,37] and the effect of electron release from the nitrogen to the electron attracting CO group.[38] Characteristic bands pointing toward 3-substitution[37,39] were present at 1095 and 762 $cm^{-1}$. However, **9** does not have a medium or strong band near 1550 $cm^{-1}$. This band is postulated[36] to arise from a lowering of the C=C frequency due to electron drift from the indole nitrogen to the 3-carbonyl. The lack of this band in **9** indicates that the band is more likely due to a ring vibration of the indole fused ring system as a whole and is therefore subject to wide variations in position.[36]†

The hydrolysis of **9** to the acid **11** was accomplished using sodium hydroxide.[30] Treatment of **11** with sodium nitrite and acidification yielded 40% of indol-3-ylglyoxal l-oxime (**6**).

Higher yields (71–95%) of **6** are realized by the base hydrolysis of **9** followed by addition of sodium nitrite and acidification without the isolation of the intermediate β-keto acid, **11**. The formation of **6**, for which there is considerable precedent,[44–48] probably results from the formation of an α-oximino acid which spontaneously decarboxylates.[49] However, a decarboxylation[49] to form the enol of 3-acetylindole followed by rapid reaction with nitrous acid to give **6** cannot be ruled out. Spectroscopic data obtained for **6** supports the assigned structure. The IR spectrum (KBr) showed that the CO band in **6** at 1585 $cm^{-1}$ has been lowered 30 $cm^{-1}$ relative to the ester **9** by H—bonding with the oximino OH moiety. The C=N stretch of the oxime in **6** is found at 1650 $cm^{-1}$, which agrees well with the value of 1640 $cm^{-1}$ quoted for oximes in the solid state.[50] The NMR spectrum of **6** corroborated the structure (DMSO-$d_6$ was the solvent of choice since chemical shifts are not concentration-dependent in this solvent[51,52]). Support for the assignments made to the protons on the indole ring is found in the NMR spectra of indole[53] and 3-acylindoles.[54] For seven α-oximinoketones, the chemical shift for the OH proton is found to fall in the range −3·64 to −2·20 τ (DMSO-$d_6$).[52] Finally, the proton attached to the oximino moiety in phenylglyoxal dioxime has been reported at 1·48 τ.[52]

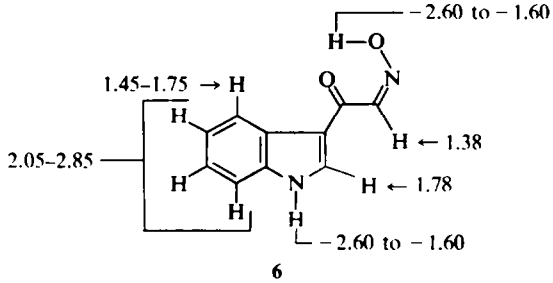
After we had completed the above synthesis, an alternative preparation *via* the nitrosation of 3-acetylindole appeared in the literature.[19] Although the reported m.p. of **6** (206°, dec) is below the one

†In the course of preparing **9**, in one instance, the indole compound **10** was obtained in 40% yield while only 9% of **9** was realized. The structure assigned **10** is supported by its IR spectrum (KBr) which showed bands at 3360, 3320, 1730, and 1695 $cm^{-1}$ indicating that two types of N–H and two types of CO are present. The UV spectra of **10** is virtually identical to that of several amino-substituted indole trimers.[40,41] Finally, the NMR spectra (DMSO-$d_6$) of **10** supplied conclusive proof of its structure. This compound, **10**, probably arises from the trimerization of indole (available due to impure methylmagnesium chloride being used) and subsequent acylation.[42,43]




**10**

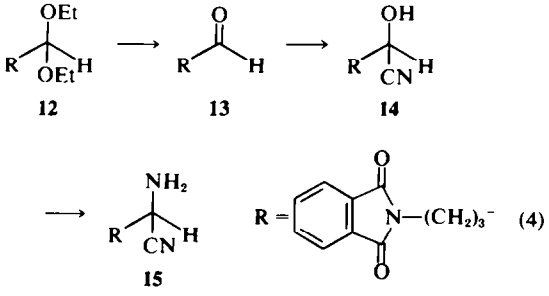
*These peaks disappear on addition of deuterium oxide.




**6**

we observed (213·5–214·5°, dec), the list of IR absorptions given[19] corresponds well with our spectrum of this material, which we obtained in 16% yield based on potassium ethyl malonate in three steps.

*2-Amino-5-phthalimido-*n*-valeronitrile p-toluenesulfonate.* The phthalimido moiety was chosen to function as the protecting group in 7.† This group ties up *both* sites on the 5-amino group and removes the possibility of ring-chain interconversion[55-57] *via* an internal condensation between the 5-nitrogen and the aldehydic carbonyl of **13**, the planned precursor of **15**. The phthalimido group is extremely acid stable,[58] yet it can be easily removed with one equivalent of hydrazine hydrate.[59]




The condensation of 4-amino-n-butyraldehyde diethyl acetal with N-(ethoxycarbonyl)-phthalimide gave **12** in 95% yield. Compound **12** was fully characterized and then subjected to mild acid hydrolysis, which gave **13** in nearly quantitative yield. Spectral data on crude **13** indicated the presence of both the phthalimido and aldehydic moieties. When the crude material was recrystallized, however, both the NMR and IR spectra showed the loss of the aldehydic group. The elemental analysis of this material indicated that $C_{12}H_{11}NO_3$ or some multiple thereof, is the correct molecular formula. The physical data indicates that the aldehyde **13** trimerized to give a trioxane derivative: 2,4,6-tri(3-phthalimidopropyl)-1,3,5-trioxane. This property of aldehydes is well known.[60] Other attempts to prepare an analytical sample of **13** were frustrated due to the unstable nature of the pure aldehyde. Since the structure of **13** is evident from the physical data, it was decided to use the crude material for the next step of the synthesis.

Attempts were made to afford a direct conversion of the aldehyde **13** to the aminonitrile **15**. The usual Strecker synthesis failed; modified homogeneous conditions using ethanol-water at pH 8 gave only the cyanohydrin. Raising the pH to 9 effected considerable decomposition (~80%) of the phthalimido ring. Attention was then turned to the preparation of **14** and its conversion to **15**, since the outlook for a direct transformation of **13** to **15** was bleak.

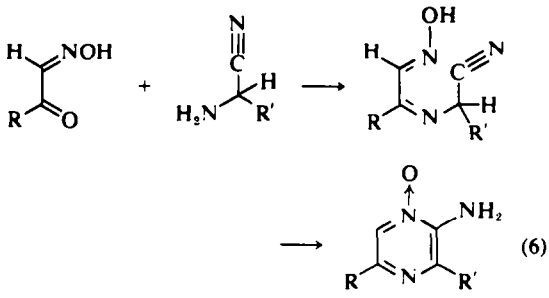
The cyanohydrin **14** was prepared in near quantitative yield from crude **13** using anhydrous liquid hydrocyanic acid with a trace of pyridine as catalyst. This method of preparing cyanohydrins is the one of choice since yields are higher and isolation easier than alkali cyanide attack on the bisulfite adduct of an aldehyde.[61-66] The IR spectrum (solid) of **14** indicated the presence of OH (3445 and 1070 $cm^{-1}$), phthalimido (1770 and 1710 $cm^{-1}$) and nitrile (weak 2240 $cm^{-1}$) functionalities. That the nitrile absorption should be weak or missing is to be expected when the C atom bonded to the nitrile group is also bonded to an electronegative group[67] such as —OH or —$NH_2$. The NMR spectrum of crude **14** indicated no **13** to be present (no absorption near 0·20 $\tau$). This crude material was used in the preparation of **15**.

Conversion of a cyanohydrin to an $\alpha$-aminonitrile usually involves basic conditions[62,68] in a solvent in which the cyanohydrin will dissociate to free aldehyde.[69-71] In our case, it was necessary to find basic conditions that would lead to aminonitrile formation but would afford only limited destruction of the base-labile phthalimide group. Therefore, the model compound N-methylphthalimide was treated with ammonia in various solvents to determine its stability. Liquid ammonia (0·5 h, −33°) or ethanol saturated with ammonia (1 h, room temp) led to greater than 80% decomposition of the model phthalimide (measured by IR spectra of the product). However, 30% aqueous ammonia (0·5 h, room temp), t-butyl alcohol saturated with ammonia (4 h, room temp), and glyme saturated with ammonia (0·5 h, 50°) gave 50% decomposition, < 20% decomposition and nearly quantitative recovery of N-methylphthalimide, respectively. It is clear that aqueous or ethanolic ammonia is unsuitable. In addition, glyme saturated with ammonia is not sufficient to dissociate the cyanohydrin **14** to any other products (90 min, room temp), an observation in accordance with the fact that dissociation of cyanohydrins in dioxane, catalyzed by amines, is extremely slow.[72] Good results however, were obtained in the conversion of **14** to **15** using t-butyl alcohol saturated with ammonia. The time course of this reaction was examined using IR spectroscopy; **14** was followed by the disappearance of the —OH stretch at 3450 $cm^{-1}$ and the phthalimide ring-opened amide was noted by the appearance of a strong band at 1660 $cm^{-1}$. The yield of **15** was determined as that of its *p*-toluenesulfonate salt.‡

†In one case, the benzoyl protecting group was used to prepare **13** (R = $-(CH_2)_3NHBz$). The NMR spectrum of this compound indicated that ring-chain interconversion was taking place and this N-protecting group was discarded in favor of the phthalimido blocking group.

‡This $\alpha$-aminonitrile *p*-toluenesulfonate salt and others similarly prepared are easily purified by recrystallization from acetonitrile. Unlike the unstable parent $\alpha$-aminonitriles, these salts are stable, non-hygroscopic and can be kept at room temperature indefinitely.

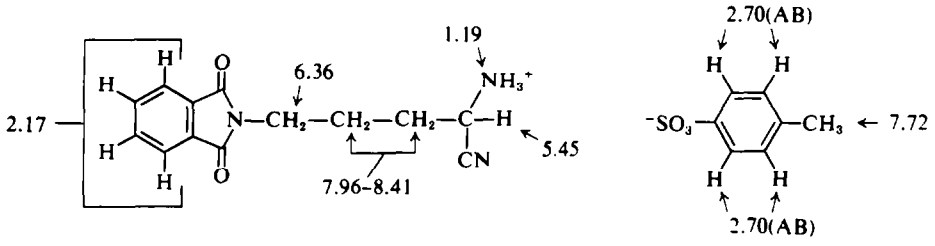
After 24 h (room temp) a small amount of **14** was present, the amount of amide was slowly increasing and the yield of **15** was 52%. Examination at 48 h showed that **14** was totally consumed the amide had increased in concentration, and the yield of **15** was 51%. At 96 h, the amide was the predominant product with the yield of **15** falling to 26%. Although the yield of **15** is virtually identical for the 24 and 48 h reaction times, the longer time is the one of choice since it gives a cleaner product. The IR spectrum of **15** was consistent with its structure, having bands at 1775 and 1715 $cm^{-1}$ (phthalimido CO), 1150 and 684 $cm^{-1}$ (—$SO_3$—), and 2700, 2650, and 1620 $cm^{-1}$ (—$NH_3^+$). There was no nitrile stretching frequency around 2220 $cm^{-1}$, but this is expected based on the reasoning given previously to account for the weakness of this band in cyanohydrins. The interpretation of the 100 mHz NMR spectrum (DMSO-$d_6$) of **15** followed from consideration of the spectra of **12** and α-aminopropionitrile *p*-toluenesulfonate. The preparation of **15** took place in 49% overall yield based on 4-aminobutyraldehyde diethyl acetal.



(6)

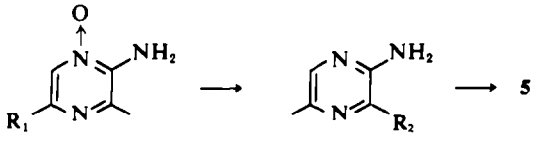
**6** is insoluble in chloroform, glyme or glyme-ethanol was used as reaction solvent). These efforts met with failure, the starting oxime being recovered in high yield (70–80%) and no material having the physical properties indicative of 2-aminopyrazine 1-oxides being found. The use of glacial acetic acid as solvent[73] did not improve matters.

The reason for these failures becomes apparent upon examination of the probable reaction mechanism shown in Eq 6. The CO moiety of **6** is deactivated and will not readily react with an α-aminonitrile in the first step of the ring forming reaction. This deactivation is due to electron drift from the indole nitrogen toward the CO group which is in a unique position to support a slight partial negative charge since it can intramolecularly H-bond with the oximino OH group. The unusually low frequency CO stretch (1585 $cm^{-1}$) of **6** also supports this contention. Therefore some reagent must be used that will not only increase the susceptability of the CO group of **6** to nucleophilic attack but will not destroy the labile α-aminonitriles.[74] Titanium tetrachloride seemed ideally suited to our purposes. This reagent has been used to prepare several enamines and imines under mild reaction conditions from ketones and amines that would not react under ordinary conditions.[75,76] It was proposed that the CO oxygen first coordinates with the titanium atom thereby preparing the CO group for reaction with amines, then the CO oxygen atom is transferred from carbon to titanium. This reagent was tested in the preparation of **19**, a known compound,[24] *via* the reaction of **17** with **18** *p*-toluenesulfonate. Free **18** was liberated by the addition of N-methylmorpholine and then the reaction was in-



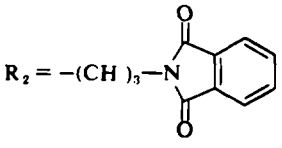
**15**

*Synthesis of 2-aminopyrazine 1-oxides.* Before utilizing **6** and **15** to prepare **16**, a direct precursor of the compound purported to be *Cypridina* etioluciferamine,[17] it was necessary to prepare several model compounds for the important spectral data they would yield. Consequently, several attempts were made to condense **6** with α-aminopropionitrile and α-aminoacetonitrile using conditions similar to those of Sharp and Spring[24,25] (since



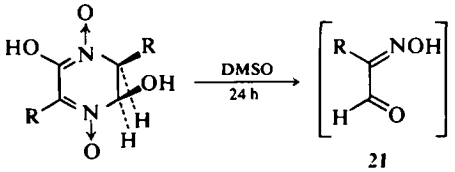
**16** **24**

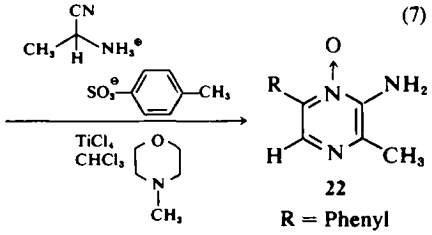
$R_1$ = indol-3-yl



(5)

itiated by the immediate addition of titanium tetrachloride. The course of the reaction was followed by the increase in extinction in the band at 350 nm (**19**) in the UV spectra of aliquots as a function of time. A yield of 40% of **19** was realized using titanium tetrachloride while only 3% of **19** was obtained by the method of Sharp and Spring (without titanium tetrachloride).[24] With or without titanium tetrachloride, the product obtained was the pyrazine **19**, showing that the metal does not cause any isomerizations during the course of the reaction which would lead to 2-aminopyrazine 1-oxides with different substitution patterns. In order to further confirm the structures of **19** it was necessary to prepare **22** and examine the spectroscopic differences between these two isomers. The pyrazine **22** was synthesized in 38% yield in a manner similar to the preparation of **19**, as shown in Eq 7. [The synthesis of **20** (by the treatment of phenylglyoxal dimethyl acetal oxime with 50% glyme – 50% pH 3·5 acetate buffer) and of **21** has been described by us previously.][77]



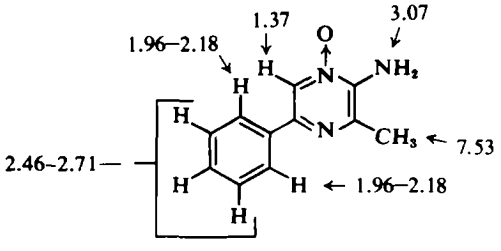



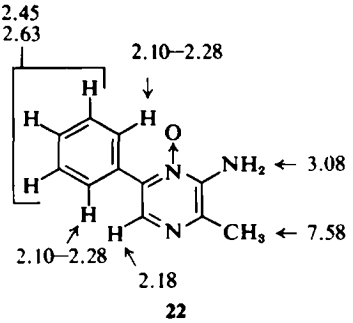

(7)

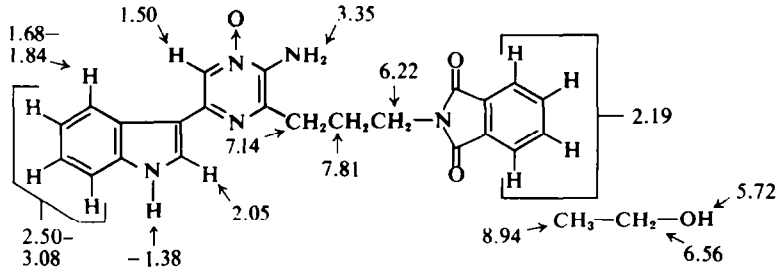
Although the UV spectra of **19** and **22** are similar, the IR spectra are completely different. However, the most important difference was found in the 100 mHz NMR spectra of these two isomers in DMSO-$d_6$ (see **19** and **22**). The marked difference of 0·81 ppm between protons when they are *ortho* or *meta* to the electron withdrawing N-oxide linkage leaves no question that the structures of **19** and **22** are correct as drawn. This evidence also demonstrated the important point that it is possible to predict the substitution pattern of a 2-aminopyrazine 1-oxide product from the structures of the starting materials. Furthermore, the large difference in chemical shift between the 5 and 6 pyrazine 1-oxide ring protons is invaluable in establishing substitution patterns where only one isomer is available.

After the successes in preparing **19** and **22** using titanium tetrachloride, the synthesis of **23** using the unreactive indol-3-ylglyoxal 1-oxime, **6**, was undertaken. Using reaction conditions similar to those used in the synthesis of **19** and **22**, a yield of 3% of **23** was obtained. This yield was raised to 44% by changing the reaction solvent from chloroform to pyridine. This dramatic yield enhancement can be explained in terms of the same effect that causes bases such as pyridine to accelerate the formation of imine products from the reaction of $Ni(en)_2Cl_2$ and acetone or acetylacetone.[78] It is postulated that the base initially coordinates with the metal and breaks the chloride bridges present. This base, now acting as a ligand, is then replaced by a CO group which undergoes subsequent condensation to form the imine complex.[78-79] The structure of **23** was confirmed by means of its NMR (DMSO-$d_6$) spectrum. The lone pyrazine ring proton of **23** is found at 1·50 $\tau$, a position which clearly shows that this is a 6-pyrazine 1-oxide proton by analogy with the model compounds **19** and **22**.

Condensation of **6** and **15** using conditions similar to those used successfully in the preparation of **23** gave only low yields (~10%) of **16**. Variations of the procedure such as raising the concentrations of reactants, the temperature, or concentration of titanium tetrachloride relative to the reactants (**6** and **15** *p*-toluenesulfonate) only resulted in the disappearance of **16** as a product of this reaction. The pattern of substitution around the pyrazine ring in **16** was confirmed by the position of the lone pyrazine ring proton in the NMR spectrum (DMSO-$d_6$). This proton is found at 1·50 $\tau$, very






**16**

similar to the position of the ring proton in the 2,3,5-substituted pyrazine 1-oxides **19** (1·37 $\tau$) and **23** (1·50 $\tau$) and quite different from the 2,3,6-substituted pyrazine 1-oxide **22** (2·18 $\tau$).

*Preparation of* Cypridina *etioluciferamine.* Although 10% Pd-C and $H_2$ gas have been used to reduce 2-aminopyrazine 1-oxides to 2-aminopyrazines,[80] this method failed in the conversion of **16** to **24** (50% ethanol, 43 h, room temp, Eq 5). A weak Raney nickel catalyst in ethanol was then used with success. Deoxygenated solvents were used to prevent formation of oxidized nickel on the catalyst surface which would then complex with **16**.† The rate of reduction was slow (82% of **24** obtained in 21 h). This is probably due to the nature of the molecule since it is known that electron-releasing groups such as methoxy or amine moieties, *ortho* or *para* to the N-oxide linkage, lead to a resistance to reduction.[81] The crude product **24** (82% yield) was contaminated with a small amount of **16**. These two compounds could not be separated by crystallization, but separation was accomplished with chromatography over silica gel (ethyl acetate as eluent). The major differences in the NMR spectra of **16** are **24** are shifts to higher field in the 6-pyrazine proton and the protons of the 2-amino moiety of **24** relative to **16**. This evidence is fully consistent with the simple deoxygenation of **16** to give **24**.

The removal of the phthalimido protecting group from **24** to give **5** was accomplished, as is usually the case,[82-84] in high yield (82%) with 100% hydrazine hydrate. A comparison was then made between the physical properties of **5** dihydrochloride and *Cypridina* etioluciferamine dihydrochloride. The IR spectra (KBr) of these two materials are virtually identical in both band position and intensity.‡[20] The UV spectra of **5** dihydrochloride and *Cypridina* etioluciferin in acidic and basic methanol are identical,§ proving that these two compounds have the same chromophore. The NMR spectrum of *Cypridina* etioluciferamine is not recorded in the literature, but the spectra (DMSO-$d_6$) of **5** dihydrochloride and etioluciferin dihydrochloride[14,85,86] are very similar. TLC data obtained from **5** dihydrochloride and *Cypridina* etioluciferamine dihydrochloride‖ showed the identity of these two compounds (Experimental). Although the m.ps of **5** dihydrochloride (263–266·5°, sealed, evacuated tube; dec) and *Cypridina* etioluciferamine dihydrochloride (251–252°, sealed tube;[17] 238–240°, sealed tube; dec)[20] are somewhat different, the close correspondence of all other physical data forces us to conclude that the two materials are identical. This identity means that the structures of *Cypridina* etioluciferamine, etioluciferin and luciferin are correct as published.[17,18]

Our synthesis of etioluciferamine constitutes a total synthesis of luciferin itself since etioluciferamine has been converted to luciferin in two steps.[18,20,85] The yield of *Cypridina* etioluciferamine obtained by us based on indol-3-ylglyoxal 1-oxime (**6**) was 8·0% (three steps) and based on 2-amino-5-phthalimido-*n*-valeronitrile *p*-toluenesulfonate (**15**) was 5% (three steps).

It is interesting to note that the structure of *Cypridina* luciferin is similar to that of *Renilla* and *Aequorea* luciferins.[87] The synthesis of *Cypridina* luciferin therefore also serves as a model for the preparation of these luciferins.

## EXPERIMENTAL

*General.* All NMR spectra were measured with a Varian A-60 Spectrometer or were run by Mr. Joseph Ahnell using a Varian HA-100 spectrometer. Chemical shifts are

†This action was prompted by the previous observation of a deep green color on the surface of the catalyst when the reduction was complete. No such color was observed when the deoxygenated solvents were used.

‡A copy of the IR spectrum of *Cypridina* etioluciferamine dihydrochloride was provided by Dr. Y. Kishi, Nagoya Univ., Chikusa, Nagoya, Japan.

§No UV spectrum of *Cypridina* etioluciferamine is recorded in the literature, but it is noted that the spectra of etioluciferamine and etioluciferin are identical.[13]

‖A sample of *Cypridina* etioluciferamine dihydrochloride was the gift of Professor Toshio Goto, Faculty of Agriculture, Nagoya Univeristy, Chikusa, Nagoya, Japan. This material was used for TLC comparison but was impure as evidenced by the presence of a second spot ($R_f$ = 0·63) on TLC (silica gel, 95% ethanol), and the fact that it melted over a wide range (208–245°, sealed, evacuated tube). A mixed m.p. with **5** dihydrochloride was therefore not attempted.

expressed on the $\tau$ scale. All IR spectra were taken on a Perkin-Elmer Model 337 spectrophotometer and calibrated with the polystyrene peak of 1601·4 $cm^{-1}$. UV spectra were recorded using a Cary Model 14 spectrophotometer using matched 1-cm quartz cells. All m.ps were taken using either a Thomas Hoover capillary m.p. apparatus or a Kofler hot stage microscope and are uncorrected. Elemental analyses were determined by Mr. Joseph Walter of The Johns Hopkins University or by Galbraith Laboratories Incorporated, Knoxville, Tennessee. N-Methylmorpholine was freshly distilled from LAH prior to use and was checked for the presence of water by means of its IR spectrum. Fluorescence emission spectra were measured on a Hitachi-Perkin-Elmer Model MPF-2A spectrophotofluorimeter. $Na_2SO_4$ was used as a drying agent unless otherwise specified.

*Ethoxycarbonylacetyl chloride*(**8**). This compound was prepared by the procedure of Gol'dfarb, *et al.*[28] The yield after distillation directly from the reaction vessel to cooled (− 60°) receivers was 74·6% [lit.[28] 68%]. This compound underwent decomposition at freezer temps; however, at dry ice temp, **8** is absolutely stable for at least 2 weeks. Distilled **8** has b.p. 38–39° (2 mm) [lit.[28] b.p. 39–40° (2 mm)]; IR (neat) 1798 and 1740 $cm^{-1}$; 100 mHz NMR (neat) $\tau$ 5·80 (q, 1·91 H, $J$ = 7·1 Hz), 6·11 (s, 1·98 H), and 8·73 (t, 3·11 H, $J$ = 7·1 Hz).

*Ethyl* 3-(*indol-3-yl*)-3-*oxopropionate* (**9**) was prepared according to the procedure of Baker, *et al.*[29] with the following exceptions. Commercial MeMgCl was used to prepare indolylmagnesium chloride. This indole Grignard reagent was then immediately added, over a period of 30 min, to a refluxing ether solution of ethoxycarbonylacetyl chloride. This mixture was refluxed an additional 45 min. A yield of 24% was realized. After recrystallization (benzene) the colorless crystals obtained had m.p. 120–121° [lit.[29] 121°]; UV max (95% EtOH) 243·5 nm (log $\epsilon$ 4·11) 261·0 (3·95), and 301·5 (4·10); IR (KBr) 3215, 1730 (ester C═O), 1615 (C═O), 1095 (indol-3-yl), and 762 $cm^{-1}$ (indol-3-yl); 100 mHz NMR (DMSO-$d_6$) $\tau$ − 2·01 (broad s, 0·94 H), 1·67 (s, 0·99 H), 1·73–1·94 (m, 0·98 H), 2·40–2·92 (m, 3·18 H), 5·90 (q, $J$ = 7·0 Hz), 6·05 (s, 3·93 H together with the quartet upon which it is superimposed), and 8·81 (t, 2·98 H, $J$ = 7·0 Hz). The dark red 2,4-dinitrophenylhydrazone derivative, prepared in the usual manner and recrystallized from acetone, had m.p. 262·5–264·0° [lit.[29] 255°].

3-(*Indol*-3-*yl*)-3-*oxopropionic acid* (**11**). This acid was obtained in 65% yield by the procedure of Oddo, *et al.*[30] and is readily decomposed (heating for 30 min at 45–50° at 12 mm pressure) to 3-acetylindole (by IR spectra comparison with purified commercial 3-acetylindole). Purified **11** has m.p. 191–192·5° [lit.[30] 192°]; UV max (95% EtOH) 242·0 nm (log $\epsilon$ 4·12), 258 (3·97), and 298 (4·12); IR (KBr) 3255 (OH), 1700 (acid C═O), and 1620 $cm^{-1}$ (C═O).

*Ethyl* o-(2,2-*diindol*-3-*ylethyl*)*anilinocarbonylacetate* (**10**). The conditions for the preparation of this compound were identical to those used in the preparation of **9** with the following exceptions. A longer reaction time was used in the preparation of indolylmagnesium chloride (60 min *vs* 40 min used in synthesis of **9**). This Grignard reagent was then added to ethoxycarbonylacetyl chloride over a period of 60 min (*vs* 30 min in the preparation of **9**). A total of 8·7% of **9** and 39·5% of **10** was obtained after the usual workup. (A mixture of these two compounds is easily separated by the addition of benzene in which **9** is soluble and **10** is not.) On recrystallization from chloroform (3x), **10** had m.p. 193–194° (crystal change 159–160°); UV max (95% EtOH) 274·5 nm (log $\epsilon$ 4·03), 282·5 (4·05) and 292·0 (4·00); IR (KBr) 3360 (indole N—H), 3320 (amide N—H), 1730 (ester C═O), 1675 (amide C═O), and 750 and 738 $cm^{-1}$ (indol-3-yl); 100 MHz NMR (DMSO-$d_6$) $\tau$ − 1·65† (s, 1·78 H), 0·64† (s, 0·80 H), 2·36–3·40 (m, 13·8 H), 5·17 (t, 1·01 H, $J$ = 7·5 Hz), 5·97 (q, 2·00 H, $J$ = 7·0 Hz), 6·47 (d, $J$ = 7·5 Hz), 6·65 (s, 4·00 H together with d at 6·47 $\tau$ upon which it is superimposed), and 8·88 (t, 3·04 H, $J$ = 7·0 Hz). Found: C, 73·73, 73·90; H 5·73, 5·79; N, 8·94. Calcd for $C_{29}H_{27}N_3O_3 \cdot 1/2\ H_2O$: C, 73·40; H, 5·95; N, 8·86.

*Indol*-3-*ylglyoxal* 1-*oxime* (**6**)

*Method A from the acid* **11**. To a soln. of 0·030 g (0·15 mmol) of **11** in 1 ml of sat $NaHCO_3$ was added 0·011 g (0·16 mmol) $NaNO_2$. The pH was then adjusted to 3 with 3% HCl to give a colorless ppt which was collected. The portion of this ppt that was insoluble in sat $NaHCO_3$(A) was then collected. This soln was then treated with approximately the same amount of $NaNO_2$ as above and taken to pH 3 as before. The resulting ppt (B) was collected and washed with water. These two solids, A and B were dried over KOH at reduced pressure ($10^{-2}$ Torr) to give 0·011 g (0·06 mmol, 40%) of a pale yellow solid. Recrystallized once from EtOH-water, this material had an IR spectrum identical to that of analytically pure indol-3-ylglyoxal 1-oxime.

*Method B from the ester* **9**. A mixture of 2·74 g (11·9 mmol) of **9** and 0·764 g (13·6 mmol) KOH in 30·5 ml distilled water was stirred at room temp for 23·5 h. At this time 0·088 g (1·56 mmol) KOH in 4 ml distilled water were added and the reaction continued for an additional 19 h. The soln was then taken to pH 7 with 3% HCl and was filtered. To the filtrate, in a cold water bath, was added 4·14 g (60 mmol) $NaNO_2$ in 8 ml distilled water followed by 20 ml 3% HCl (over a 5 min period). Evolution of a gas took place and flocculent ppt formed. This material was collected on a Büchner funnel, washed with 25 ml distilled water, and the filtrates combined. The addition of $NaNO_2$ and HCl was repeated 4 more times as follows: $NaNO_2$ (2·07 g, 30 mmol) in 14 ml $H_2O$ followed by 10 ml 3% HCl, twice and $NaNO_2$ (4·14 g, 60 mmol) in 18 ml $H_2O$ followed by 20 ml 3% HCl, twice. The ppt that formed after every set of additions was collected and washed with water, the filtrates then being combined prior to the next set of additions. The ppts were combined and dried at reduced pressure over KOH to give 2·04 g (10·8 mmol, 92%) of an off-white powder. An IR spectrum of this material gave a peak for peak correlation with that of analytically pure indol-3-ylglyoxal 1-oxime.

A sample of indol-3-ylglyoxal 1-oxime was recrystallized 3 times from EtOH-water: m.p. 213·5–214·5° (dec) [lit.[19] 206° (dec)]; UV max (95% EtOH) 237·5 nm (log $\epsilon$ 4·03), 251·0 (4·02), 270·0 (3·96), 276·0 (3·93), and 332·5 (4·01); IR (KBr) 3230 (indole N—H and oxime O—H), 1650 (oxime C═N), and 1585 $cm^{-1}$ (C═O); NMR (DMSO-$d_6$) $\tau$ − 2·60 to − 1·60 (broad s, 1·51 H), 1·38 (s, 0·96 H), 1·45–1·75 (m, 0·94 H), 1·78 (s, 1·03 H), and 2·05–2·85 (m, 3·08 H). Found: C, 63·84; H, 4·31; N, 14·89. Calcd for $C_{10}H_8N_2O_2$: C, 63·83; H, 4·28; N, 14·89.

4-*Phthalimido*-n-*butyraldehyde diethyl acetal* (**12**). To a soln of 16·0 g (0·190 mol) $NaHCO_3$ in 285 ml distilled water were added 30·6 g (0·190 mol) 4-amino-n-butyraldehyde diethyl acetal and 43·6 g (0·199 mol) N-(ethoxycarbonyl)phthalimide. This suspension was stirred

†Disappears on addition of deuterium oxide.

at room temp for 1·5 h. The mixture was then extracted (5×250 ml) with $CCl_4$ that had stood over $Na_2CO_3$ for one week. The combined $CCl_4$ extracts were then extracted (7×250 ml) with 2·5% $NaHCO_3$ (pH 8). After drying over anhyd $Na_2CO_3$, the $CCl_4$ soln was filtered and the solvent removed at reduced pressure to give 52·5 g (0·18 mol, 95%) of light yellow oil. Upon scratching, this oil crystallized to an off-white solid that had m.p. 34–41°. The IR spectrum of this material was identical in all respects with that of an analytically pure sample of 4-phthalimido-n-butyraldehyde diethyl acetal prepared by sublimation (70°, $10^{-2}$ Torr) which had m.p. 40–41°; UV max (95% EtOH) 232·0 nm (log $\epsilon$ 4·13), 238·5 (3·97), and 291·0 (3·27); IR (KBr) 1770 and 1710 (phthalimido C=O), 1123 and 1060 (acetal C—O), and 722 $cm^{-1}$ (aromatic out-of-plane); NMR ($CCl_4$) $\tau$ 2·24 and 2·27 (two s, 4·02 H), 5·38–5·67 (m, 0·96 H), 6·17–6·83 (m, 6·10 H), 8·10–8·55 (m, 4·12 H), and 8·85 (t, 6·18 H, $J$ = 7·2 Hz). Found: C, 65·86; H, 7·32; N, 4·72. Calcd for $C_{16}H_{21}NO_4$: C, 65·96; H, 7·27; N, 4·81.

*4-Phthalimido-n-butyraldehyde* (**13**). To a soln of 2·72 g (9·36 mmol) of **12** in 20 ml reagent dioxane was added 3 ml distilled water followed by 3 ml 1 N HCl. This homogeneous soln was stirred at room temp under $N_2$. After 2 h, the reaction was stopped by the slow addition of 3 ml 1 N $NaHCO_3$. The solvents were removed at reduced pressure (water aspirator). The resulting oil and solid were extracted with 50 ml chloroform. This extract was dried over $Na_2SO_4$ and taken to dryness at reduced pressure to give 2·14 g (9·88 mmol, 105%) of a pale yellow oil which spontaneously crystallized (colorless needles) after one day in the freezer. This material had m.p. 60–68·5°; IR ($CHCl_3$) 2820 and 2720 (aldehyde C—H), and 1770 and 1710 (phthalimido and aldehydic C=O); NMR ($CDCl_3$) $\tau$ 0·20 (t, 0·83 H, $J$ = 1·0 Hz), 2·17 and 2·20 (two s, 3·98 H), 6·23 (t, 2·46 H, $J$ = 6·7 Hz), 7·42 (degenerate t, 1·76 H, $J$ 7·0 Hz), and 7·93 (quintet, 1·98 H, $J$ = 7·0 Hz).

An analytical sample of this material was not prepared since recrystallization led to 2,4,6-tri(3-phthalimidopropyl)-1,3,5-trioxane. Sublimation (70° and $10^{-2}$ Torr) gave a colorless solid, m.p. 52·5–68·5°, which rapidly underwent decomposition, even under $N_2$, to become off-white and sticky.

2,4,6-*Tri*(3-*phthalimidopropyl*)-1-3,5-*trioxane*. A sample of crude **13** (obtained as detailed above) was extracted with diethyl ether. The remaining residue was recrystallized from acetone-water 3 times to give colorless needles with m.p. 146–149° (very slow increase in stage temp) or 124–136° (fast increase) with a crystal change at 102°; UV max (95% EtOH) 232·0 nm (log $\epsilon$ 4·12) 240·5 (3·95), and 291·0 (3·31); IR (KBr) 1775 and 1710 (phthalimide C=O) and 720 $cm^{-1}$ (aromatic out-of-plane); 100 mHz NMR ($CDCl_3$) $\tau$ 2·06–2·48 (m, 4·09 H), 5·10 (t, 1·05 H, $J$ = 4·4 Hz), 6·20–6·56 (m, 1·95 H), and 7·96–8·60 (m, 3·86 H). Found: C, 66·04; H, 5·03; N, 6·30. Calcd for $(C_{12}H_{11}NO_3)_3$: C, 66·35; H, 5·10; N, 6·45.

2-*Hydroxy*-5-*phthalimido*-n-*valeronitrile* (**14**). To 23·4 g (0·108 mol) of crude **13** was 0·5 ml pyridine (dried over KOH pellets) in a cooled (0–3°) reaction flask fitted with a dry ice condenser was introduced approximately one mole of anhyd HCN gas. This gas was generated by dripping a soln of 51·0 g (1·04 mol) of NaCN in 85·0 ml of distilled water into a soln of 98·0 (1·00 mol) $H_2SO_4$ in 53·5 ml distilled water. Drying of the gas was effected by passing it through a heated (hot water bath, 50°) 100 mm U-tube packed with anhyd $CaCl_2$ prior to condensation in the reaction flask. When all the gas had been generated, the homogeneous reaction soln stirred at 0–3° for 3 h after which the hydrocyanic acid was slowly distilled out at reduced pressure (12 Torr then $10^{-2}$ Torr). In this manner, 24·5 g (0·100 mol, 93%) of 2-hydroxy-5-phthalimido-n-valeronitrile were obtained. No attempt was made to obtain an analytically pure sample of this compound. The structural features of the molecule were revealed by spectra of the crude material prepared above: IR (solid film) 3445 (O—H), 2240 (C≡N, weak), 1770 and 1710 (phthalimido C=O), 1070 (C—O), and 722 $cm^{-1}$ (aromatic out-of-plane); NMR ($CDCl_3$) 2·22 (s, 3·98 H), 3·80–4·60 (broad s, 1·03 H), 5·05–5·50 (m, 0·97 H), 5·90–6·60 (m, 2·39) and 7·80–8·50 (m, 2·39 H). There was no absorption in the region −0·50 to 1·00 $\tau$ (aldehydic C—H).

2-*Amino*-5-*phthalimido*-n-*valeronitrile* p-*toluenesulfonate* (**15**). A rapid stream of anhyd ammonia was passed, for 7 mins, over a stirred soln of 1·58 g (6·48 mmol) crude **14** in 50 ml reagent t-BuOH at room temp. The reaction flask was fitted with a gas inlet tube and a drying tube packed with anhyd $CaCl_2$. This procedure was repeated at 12, 24, and 36 h reaction times, in between which times, the reaction was stirred at room temp. After a total of 48 h, the soln was transferred to a 3 l flask. The t-BuOH was solidified on the walls of the flask in a thin film (ice-bath) and was removed along with the ammonia at reduced pressure ($10^{-2}$ Torr for 1·5 h at room temp). The pale yellow intractable oil thus obtained was treated first with 20 ml chloroform, then with 60 ml anhyd diethyl ether. After standing for 15 min, the soln was decanted from the ppt which formed, which was then washed (2×10 ml) with anhyd ether. The washings were combined with the chloroform ether soln and the solvents were removed at reduced pressure. The resultant oil was dissolved in 5 ml of chloroform and 0·851 g (4·48 mmol) *p*-toluenesulfonic acid monohydrate in 40 ml of anhyd ether were added. After standing 15 min, the liquid was decanted and the ppt washed with anhyd ether. After drying at reduced pressure, 1·40 g (3·39 mmol, 52%) of nearly colorless material was obtained whose IR spectrum was virtually identical to that of an analytically pure sample of 2-amino-5-phthalimido-n-valeronitrile *p*-toluenesulfonate prepared by recrystallization from acetonitrile (3x). The purified material (tiny colorless needles) had m.p. 169·5–170·5° (decomp); UV max (95% EtOH) 233·0 nm (log $\epsilon$ 4·17), 238·0 (3·98), 241·0 (4·00), 257·0 (2·83), 262·0 (2·92), 268·0 (2·97), and 293·5 (3·31); IR (KBr), 2700, 2650, and 1620 (—$NH_3^+$), 1775 and 1715 (phthalimido C=O), 1150 and 684 (—$SO_3$—), 1028, 1005, and 813 (aromatic 1,4-substitution) and 720 $cm^{-1}$ (aromatic 1,2-substitution); 100 mHz NMR (DMSO-$d_6$) $\tau$ 1·19 (broad s, 2·48 H), 2·17 (s, 3·96 H), 2·70 (AB q, 4·23 H), 5·45 (t, 0·92 H, $J$ = 6·5 Hz), 6·36 (t, 2·00 H, $J$ = 6·0 Hz), 7·72 (s, 2·97 H), and 7·96–8·41 (m, 3·80 H). Found: C, 58·51, 58·54; H, 5·28, 5·06; N, 10·07; S, 7·70. Calcd for $C_{20}H_{21}N_3O_4S$: C, 57·82; H, 5·10; N, 10·11; S, 7·72. Another analyst found on the same sample: C, 58·04; H, 5·27.

*α-Aminopropionitrile* p-*toluenesulfonate* (**18**). α-Aminopropionitrile was prepared by the method of Kendall and McKenzie.[88] The *p*-toluenesulfonate salt was prepared by adding an ether soln of *p*-toluenesulfonic acid monohydrate to an ether solution of α-aminopropionitrile. A yield of 52% was obtained. Recrystallization twice from acetonitrile gave colorless needles: m.p. 169·5–170·5° (dec); UV max (95% EtOH) 253 nm (log $\epsilon$ 2·24), 258 (2·36), 263·5 (2·42), and 270 (2·29); IR (KBr) 2700, 2600, 2515, and 2070 (—$NH_3^+$), 2250 (very weak,

—C≡N), 1170, and 687 (—$SO_3^-$), and 1035, 1010, and 816 $cm^{-1}$ (aromatic 1,4-substitution); NMR (DMSO-$d_6$) $\tau$ 1·71† (broad s, 2·72 H), 2·89 (AB q, 4·09 H) 5·57 (q, 1·00 H, $J$ = 7·0 Hz), 7·78 (s, 2·97 H), and 8·51 (d, 3·03 H, $J$ = 7·0 Hz). (Found: C, 49·62; H, 5·88; N, 11·57. Calcd for $C_{10}H_{14}N_2O_3S$: C, 49·57; H, 5·82; N, 11·56).

*2-Amino-3-methyl-5-phenylpyrazine 1-oxide* (**19**)

*Method A without titanium tetrachloride.* A yield of 3% of **19** was obtained using the method of Sharp and Spring.[24] This material had m.p. 188·5–189·5° (dec) [lit.[24] 188–189°]. An analytical sample was prepared by sublimation (180°/5 × $10^{-2}$ Torr) and subsequent washings with cold MeOH. The colorless solid thus obtained had m.p. 189·5–190·5°; UV max (95% EtOH) 261·0 nm (log $\epsilon$ 4·40), 286·5 (4·17), and 350·5 (3·80); IR (KBr) 3320, 3250, and 3100 ($H_2O$ and N—H), and 913 $cm^{-1}$ (N→O); 100 mHz NMR (DMSO-$d_6$) $\tau$ 1·37 (s, 0·95 H), 1·96–2·18 (m, 2·02 H), 2·46–2·71 (m, 3·18 H), 3·07 (broad s, 1·84 H), 6·73 (s, 5·90 H), and 7·53 (s superimposed on dimethyl sulfoxide multiplet). Found: C, 63·59; H, 5·61; N, 20·21. Calcd for $C_{11}H_{11}N_3O \cdot 1/3\ H_2O$: 63·77; H, 5·67; N, 20·28.

*Method B with titanium tetrachloride.* A mixture of 0·228 g (1·53 mmol) $\omega$-isonitrosoacetophenone and 1·22 g (5·04 mmol) **18** in 3·1 ml dry chloroform was prepared (the reactants were previously dried at room temp and $10^{-2}$ Torr for 2·5 h). Injections (*via* syringe) of 85 mg (0·84 mmol) of N-methylmorpholine were made very 6 h through a rubber septum into this stirred reaction mixture. Each injection was immediately followed by another consisting of 55 mg (0·29 mmol) titanium tetrachloride in 0·1 ml dry chloroform. Five sets of injections were made. After 57 h, the deep red reaction mixture was diluted with 10 ml chloroform and filtered. The ppt was washed with 40 ml chloroform, the filtrates combined and the solvent removed at reduced pressure. The red oil thus obtained was extracted (3 × 10 ml) with 2 N HCl. These extracts were combined (A). The remaining red oil was then treated with 2 ml of 2N NaOH, which converted it into a brown solid (B). After 1 h, this mixture was diluted with 5 ml of distilled water and filtered. The ppt (B) was first washed with 25 ml distilled water then dissolved in acetone. The solvent was removed at reduced pressure to give 27 mg (0·13 mmol, 9%) solid material. The combined liquids from the HCl extracts (A) were taken to pH 8 with 2 N NaOH. The resultant ppt was collected, washed with 10 ml of water (filtrates combined (C)) and dissolved in 50 ml acetone. The acetone soln was dried ($Na_2SO_4$) and the solvent removed at reduced pressure to give 33 mg (0·16 mmol, 10%) of yellow solid. Crystals slowly grew in the combined filtrates (C). These were collected, washed with 10 ml distilled water and the filtrates combined (D). These crystals weighed 14 mg (0·07 mol, 5%). These combined filtrates (D) were freeze-dried to give a yellow solid. This material was treated with 10 ml of 2 N NaOH, allowed to set overnight, then freeze-dried again. After treatment of the resultant yellow solid with 5 ml of water, the insoluble material was collected, washed (3 × 15 ml) with distilled water and dried to give 50 mg (0·25 mmol, 16%) of solid material. *Via* these physical separations, 124 mg (0·61 mmol, 40%) of **19** were obtained in four fractions. The IR spectra of these materials showed them to be identical to one another and to an analytically pure sample of 2-amino-3-methyl-5-phenylpyrazine 1-oxide.

†Disappears on addition of deuterium oxide.

*2-Amino-3-methyl-6-phenylpyrazine 1-oxide* (**22**). To phenylglyoxal 2-oxime (**17**), obtained by treatment of 745 mg (2·50 mmol) of **20** with 1·5 ml dimethyl sulfoxide,[77] was added 3·99 g (16·5 mmol) of **18**. This mixture was dried at reduced pressure ($10^{-2}$ Torr at room temp for 1 h), the flask was flushed 3 times with dry $N_2$ then capped with a rubber serum stopper. An injection (*via* syringe) of dry chloroform (10 ml) was followed by another of 1·84 ml (1·66 g, 16·5 mmol) dry N-methylmorpholine. One minute later, 0·63 ml (1·09 g, 5·72 mmol) titanium tetrachloride were injected. The progress of the reaction was followed by removing measured aliquots of the reaction soln and observing the increase in optical density at 343 nm (95% EtOH) as a function of time. After 84 h, 50 ml of 95% EtOH were added to the reaction solution. The solvents were then removed at reduced pressure (water aspirator followed by $10^{-2}$ Torr pressure at room temp for 9 h) to give 8·79 g of a deep red oil. This material was adsorbed onto 30 g of Woelm alumina for dry column chromatography (No. 103) and was dry column chromatographed over 350 g of the same substance using 90% EtOH as the eluent. The first 450 ml contained **22** (by UV spectra); accordingly, the solvent was removed at reduced pressure to give 1·02 g of a red gum. This material was spotted onto six 20 × 20 cm preparative silica gel plates [each prepared from 20 g of silica gel G, 0·20 g of Baker fluorescent indicator and 40 ml of water, then oven dried (100°, 4 h), and deactivated (open to air for 1 h)] and eluted with EtOAc. The bands falling between $R_f$ 0·11 and 0·42 (average values) were removed and washed with 95% EtOH. After removal of this solvent at reduced pressure, 379 mg (0·188 mmol, 38%) of very pale yellow crystals were obtained. The IR spectrum of this crude material was virtually identical to that of an analytically pure sample of **22** prepared by sublimation (158°/5 × $10^{-2}$ Torr) and subsequent washing with anhyd MeOH. The colorless micro-crystals thus obtained had m.p. 172·5–174·5°; UV max (95% EtOH) 242·0 nm (log $\epsilon$ 4·28), 292·5 (3·63, shoulder), and 343·0 (3·97); IR (KBr) 3050, 3265, and 3180 (—$NH_2$), and 897 and 883 $cm^{-1}$ (N→O); 100 mHz NMR (DMSO-$d_6$) $\tau$ 2·10–2·28 (m), 2·18 (s, 3·06 H together with previous multiplet), 2·45–2·63 (m, 2·96 H), 3·08† (broadened s, 1·99 H), and 7·58 (s, superimposed on the multiplet of DMSO). Found: C, 65·89; H, 5·46; N, 20·98. Calcd for $C_{11}H_{11}N_3O$: C, 65·66; H, 5·51; N, 20·88.

*2-Amino-5-(indol-3-yl)-3-methylpyrazine 1-oxide* (**23**). A soln of 124 mg (0·66 mmol) of **6** and 527 mg (2·18 mmol) of **18** in 2 ml dry pyridine was prepared under $N_2$ in a small flask fitted with a rubber septum. In rapid succession, 0·08 ml (approx 139 mg, 0·73 mmol) of titanium tetrachloride and 0·25 ml (approximately 230 mg, 2·18 mmol) of N-methylmorpholine were injected *via* syringe into this soln. The reaction proceeded with stirring at room temp and was followed by periodically removing 2 $\mu$l samples from the deep red reaction solution and chromatographing them on alumina strips (Eastman No. 6062) using 90% EtOH as the eluent. The highly fluorescent green streak, found between $R_f$ 0·26 and 0·68 was removed and put into 10 ml of 95% EtOH. Fluorescence emission spectra (380–580 nm with an excitation wavelength of 366 nm) of these samples were compared to that of a standard solution of 2-amino-5-(indol-3-yl)-3-methylpyrazine 1-oxide (previously prepared). The course of the reaction was followed by comparison of peak heights at 464 nm of samples and standard. After 48·5 h, the reaction was quenched with 10 ml of 95% EtOH and volatile components were then removed under reduced pressure (water

aspirator followed by 0·5 h at $10^{-2}$ Torr pressure and 45°). The remaining red oil was adsorbed onto Woelm neutral alumina and chromatographed over 200 g of this material using the dry column technique (90% EtOH as eluent). The strongly yellow-green fluorescent band ($R_F$ 0·43–0·68) was removed and the compound washed off the alumina with 25 ml of 95% EtOH followed by 200 ml of 90% EtOH. After removing this solvent at reduced pressure, the remaining solid was extracted with 30 ml of acetone. After drying the soln over $Na_2SO_4$, the acetone was removed to give 70 mg (0·29 mmol, 44%) of a crystalline solid that gave an IR spectrum (KBr) virtually identical to that of 2-amino-5-(indol-3-yl)-3-methylpyrazine 1-oxide prepared previously. After two recrystallizations from EtOH-water, the straw colored crystals had m.p. 229–230° (dec); UV max (95% EtOH) 275·5 nm (log ε 4·22), 298·0 (4·19), and 372·0 (3·66); IR (KBr) 3625–3000 ($H_2O$, N—H and $NH_2$) and 890 $cm^{-1}$ (N→O); 100 nHz NMR (DMSO-$d_6$) $\tau$ – 1·40 (broadened s, 1·00 H), 1·50 (s, 0·96 H), 1·64–1·86 (m, 1·25 H), 2·07 (d, 1·05 H, $J$ = 2·5 Hz), 2·50–3·10 (m, 3·30 H), 3·40 (broadened s, 1·37 H), 6·69 (s, $H_2O$ not integrated), and 7·50 (s, superimposed on multiplet of DMSO). Found: C, 62·70; H, 4·88; N, 22·32. Calcd for $C_{13}H_{12}N_4O \cdot 1/2\ H_2O$: C, 62·64; H, 5·26; N, 22·48.

*2-Amino-5-indol-3-yl-3-(3-phthalimidopropyl) pyrazine 1-oxide* (**16**). To a cooled soln (18–19°) of 1·93 g (10·2 mmol) of **6** and 7·02 g (16·9 mmol) of **15** in 30·2 ml dry pyridine, under dry $N_2$, in a flask fitted with a rubber septum was added (*via* syringe) 1·25 ml (11·3 mmol) titanium tetrachloride. Immediately thereafter, an injection of 1·89 ml (16·9 mmol) N-methylmorpholine was made. The stirred reaction soln kept at 18–19° became orange, then deep red in colour.

Aliquots were taken at intervals and subjectd to TLC on silica gel sheets (Eastman No. 6060) using EtOAc as the eluent. By scraping off the portions $R_f$ 0·03→0·10 (previously shown to be the desired product) and $R_f$ 0·10→0·40 (the starting α-oximinoketone **6**), adding these to equal volumes of 95% EtOH (5 ml), and taking UV spectra of these solns, the course of the reaction could be followed. No change in these UV spectra occurred between 18 and 28 h and the starting material **6** had largely disappeared. The reaction was stopped after 29·5 h by adding the deep red soln to 300 ml 95% EtOH. The soln was then filtered from the brown ppt that separated and this ppt was washed (6×50 ml) with 95% EtOH. The combined filtrate and washings were taken to dryness at reduced pressure (water aspirator) and the resultant brown oil was treated with 250 ml distilled water. The solid that slowly separated was collected, washed (2×50 ml) with distilled water and dried overnight (room temp and $10^{-2}$ Torr) to give 2·80 g material. It was shown in a separate experiment with N-methylphthalimide that although the phthalimide ring is destroyed by being kept over alumina (95% EtOH) for long periods of time (10% recovery of N-methylphthalimide after 139 h), this decomposition isn't a serious problem in chromatographies of short duration (81% recovery after 9 h). Therefore, 0·5 g of the crude reaction product (2·80 g total) was dry-column chromatographed over 69 g of activity III alumina using 0·1% of a fluorescent indicator in a glass column 1·8 cm in diameter using 1:1 EtOAc-95% EtOH as the eluent. The desired product **16** moved slowly as a pale green fluorescent band (under long wave UV light). The fractions that gave a single spot on TLC and that had an UV spectrum indicating that the desired pyrazine 1-oxide was present were combined and the solvent removed at reduced pressure to give 73 mg of a yellow oil that slowly crystallized.

The remainder of the crude brown reaction product (2·30 g) was then chromatographed in a manner similar to that just described (400 g of activity III alumina with 0·1% fluorescent indicator in a 4·5 cm diameter column using 1:1 EtOAc-95% EtOH as the eluent) to give 385 mg of a yellow oil that slowly crystallized. Thus, from the two chromatographies, a total of 458 mg (1·11 mmol, 11%) crude **16** was obtained. This compound is best crystallized by dissolving it in a large volume (400 mg/200 ml) EtOH using occasional warming in a hot water bath (~50°) to aid soln (extensive heating is to be avoided as this leads to decomposition of this compound), then slowly removing the solvent at the water aspirator (using a room temp water bath) until the soln becomes turbid. In this manner, after two crystallizations, beautiful clusters of yellow needles were obtained on standing: m.p. 225–228° (dec); UV max (95% EtOH) 231·0 nm (log ε 4·59), 240·0 (4·44), 277·0 (4·31), 297·5 (4·33), and 378·0 (3·68); IR (KBr) 3650–2975 (indole N—H, EtO—H, and pyrazine $NH_2$; as the pellet picks up water and becomes cloudy, three sharp bands at 3445, 3370, and 3320 $cm^{-1}$ emerge), 1770 and 1700 (phthalimido CO), 887 (N→O), 743 (aromatic out-of-plane, indole), and 719 $cm^{-1}$ (aromatic out-of-plane, phthalimido); 100 mHz NMR (DMSO-$d_6$) $\tau$ – 1·38† (broad s, 0·92 H), 1·50 (s, 1·06 H), 1·68–1·84 (m, 1·13 H), 2·05 (d, 1·13 H, $J$ = 2·2 Hz, collapses to s on addition of $D_2O$ to the sample), 2·19 (s, 3·72 H), 2·50–2·73 (m, 1·13 H), 2·73–3·08 (m, 2·13 H), 3·35† (broadened s, 2·03 H), 5·72† (t, 0·90 H, $J_{CH_2}$ = 5·0 Hz), 6·22 (t, 2·13 H, $J_{CH_2}$ = 7·0 Hz), 6·56 (d of quartets, 1·97 H, $J_{OH}$ = 5·0 Hz, $J_{CH_3}$ = 7·0 Hz, collapses to a single quartet, $J_{CH_3}$ = 7·0 Hz, on addition of $D_2O$ to the sample), 6·73† (s, 2·36 H), 7·14 (t, 2·30 H, $J_{CH_2}$ = 7·5 Hz), 7·81 (quintet, 2·46 H, $J_{CH_2}$ = 7·0 Hz), and 8·94 (t, 2·71 H, $J_{CH_3}$ = 7·0 Hz). Found: C, 64·34; H, 5·38; N, 1503, 15·15. Calcd for $C_{23}H_{19}N_5O_3 \cdot C_2H_5OH \cdot 1/2\ H_2O$: C, 64·09; H, 5·59; N, 14·95.

*2-Amino-5-indol-3-yl-3-(3-phthalimidopropyl) pyrazine* (**24**)

*A. Preparation of weakly active Raney nickel catalyst.* To an ice-cooled soln of 45 ml of 20% NaOH was added 5 g Raney Ni alloy catalyst powder in small portions over a 5 min period. After an additional 5 min in the ice bath, the mixture was heated to 100–110° for 1 h with occasional stirring. Cooling for 5 min at room temp was then followed by adding 100 ml distilled water to the mixture, mixing, and decanting the liquid after the black powder had settled. Both the water and EtOH used in the preparation of the catalyst and in the reduction described below were brought to a boil, covered, and allowed to cool to room temp. They were used immediately. The washing procedure was repeated 29 times with water and 5 times with abs EtOH (50 ml each time with this solvent). The pH of the wash liquor was no longer basic after 15 of the above described water washings (pH 6). The Raney Ni thus prepared was stored in abs EtOH under $N_2$ in the freezer.

*B. Synthesis* via *Raney* Ni. To a soln of 177 mg (0·38 mmol) of **16** in 70 ml of abs EtOH at room temp was added approximately 300 mg (2·5 g-atom) Raney Ni catalyst sludge. $H_2$ gas was bubbled vigorously through the stirred soln by means of a glass pipette; EtOH was added periodically to maintain the starting volume. After 9 h, an additional 300 mg of the catalyst sludge was added since

†Disappears on addition of deuterium oxide.

TLC (Eastman No. 6060 silica gel sheets, EtOAc as eluent) indicated that the reaction was proceeding slowly. After 21 h, the soln was warmed to 50° (to dissolve product on sides of vessel), filtered and the black solid washed (5 × 10 ml) with warm abs EtOH. The filtrates were combined and the solvent removed at reduced pressure to give 82 mg of a yellow solid. The recovered catalyst was then washed (5 × 5 ml) with DMSO. After combination of filtrates and solvent removal, 54 mg of yellow material were isolated. TLC's of these two yellow solids showed them to be identical to one another. IR spectra of these materials were very similar to each other and to that of an analytically pure sample of **24** previously prepared. However, a 100 mHz NMR spectrum (DMSO-$d_6$) showed that the reaction product was contaminated with 10% (by integration) of the starting N-oxide **16** (no other contaminant present). The crude yield of **24** is therefore 122 mg (0·31 mmol, 82%) and the recovery of **16** is 14 mg (0·03 mmol, 8% recovery). The N-oxide **16** could not be separated from **24** by recrystallization (abs MeOH), however the two compounds were resolved by dry column chromatography (Merck silica gel PF 254 which contained 0·5% Baker No. 2100 fluorescent indicator, EtOAc as the eluent). The material which moved close to the solvent front was eluted from the column and recrystallized once from abs MeOH to give an analytically pure sample of **24**: m.p. 245·5–248·0° (dec): UV max (MeOH) 231·5 nm (log $\epsilon$ 4·54, shoulder), 240·5 (4·32, shoulder), 273·0 (4·26), 289·0 (4·22) and 362·0 (3·87); IR (KBr) 3470 and 3370 (indole N—H and pyrazine $NH_2$), 1780, 1763 and 1700 (phthalimido C=O), 737 (aromatic out-of-plane, indole) and 719 and 710 $cm^{-1}$ (aromatic out-of-plane, phthalimido); 100 mHz NMR (DMSO-$d_6$) $\tau$ −1·25† (broadened s, 1·04 H), 1·72 and 1·68–1·88 (s, superimposed on a multiplet, 2·01 H), 2·15 and 2·17 (small s, large s, 4·62 H), 2·52–2·63 (m, 1·10 H), 2·78–3·10 (m, 2·12 H), 4·10† (broadened s, 1·82 H), 6·21 (t, 1·97 H, $J$ = 7·0 Hz), 6·72† (s, 3·41 H), 7·25 (t, 2·04 H, $J$ = 7·0 Hz) and 7·82 (quintet, 2·04 H, $J$ = 7·0 Hz). Found: C, 69·62; H, 5·00. Calcd for $C_{23}H_{19}N_5O_2$: C, 69·51; H, 4·82.

*2-Amino-3-(3-aminopropyl)-5-indol-3-ylpyrazine* (Cypridina *etioluciferamine*, **5**). To a stirred soln of 50 mg (0·126 mmol) of **24** in 10 ml abs EtOH at room temp was added 20 mg (0·40 mmol) 99–100% hydrazine hydrate. The reaction proceeded very slowly as evidenced by TLC data (Eastman alumina sheets No. 6063 using abs EtOH as eluent). Accordingly, 20 mg (0·40 mmol) 99–100% hydrazine hydrate were added at 22·5 h and an additional 40 mg (0·80 mmol) of this reagent at 28 h total reaction time. After 43 h, the soln was taken to dryness (water aspirator, then $10^{-2}$ Torr) taking care to remove the last traces of hydrazine hydrate. This treatment gave 60 mg of a yellow solid which was treated with 2·5 ml 0·5 N HCl. After placing the resulting yellow soln and off-white ppt in an ice bath for 2 h, the mixture was filtered and the ppt washed (9 × 1 ml) with 0·5 N HCl. The filtrates were combined and freeze-dried to give 46 mg of yellow solid which was recrystallized (MeOH) to give 35 mg (0·103 mmol, 82%) in two crops. The material from the first crop was recrystallized (MeOH) again to give yellow microcrystals that had m.p. 263–266·5° (sealed, evacuated tube, dec) [lit.[20] 238–240° (dec)]; UV max (MeOH—0·1 N NaOH) 226·5 nm (log $\epsilon$ 4·35), 272·5 (4·28), 288·0 (4·19 shoulder), and 361·5 (3·90); (MeOH—0·1 N HCl) 221·0 (4·37), 275·0 (4·17), 305·0 (4·28), and 403·5 (3·63); IR (KBr) 3445, 3288, 2977, 1645, 1620, 1544, 1523, 1480, 1452, 1438, 1425, 1380, 1333, 1271, 1238, 1176, 1126, 1010, 954, 840, 764, 744, 665, 620, 607, 577, and 554 $cm^{-1}$; 100 mHz NMR (DMSO-$d_6$) $\tau$ − 1·68 (broadened s, 0·92 H), 1·40 (broad s), 1·68 (s), 1·60–1·90 (m), 1·97 (d, 9·32 H for this and previous peaks with no integration given, $J$ = 2·5 Hz), 2·44–2·66 (m, 1·06 H), 2·72–2·98 (m, 2·00 H), 7·00 (degenerate t, 3·65 H, $J$ = 7·2 Hz), and 7·84 (quintet, 1·98 H, $J$ = 7·6 Hz); (DMSO-$d_6$ and $D_2O$) 1·58 (s), 1·50–1·77 (m), 1·81 (s), 2·18–2·43 (m), 2·45–2·78 (m), 6·78 and 6·85 (two overlapping t, $J_{C_3H—C_2H}$ = 7·0 Hz, $J_{C_1H—C_2H}$ = 8·0 Hz), and 7·62 (quintet); TLC [support (Eastmen sheets with fluorescent backing), eluent, $R_f$ of **5** dihydrochloride, $R_f$ of *Cypridina* etioluciferamine dihydrochloride] silica gel, 95% EtOH, 0·17, 0·18; silica gel, 95% EtOH, then acetone, 0·22, 0·24; silica gel, 50% MeOH, 0·15, 0·15; alumina, 50% MeOH, 0·32, 0·34. Found: C, 46·56; H, 6·22. Calcd for $C_{15}H_{17}N_5 \cdot 2HCl \cdot 5/2\ H_2O$: C, 46·76; H, 6·28.

†Disappears on addition of deuterium oxide.

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