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Synthesis and Activity of 3-(1-Alkylaminoalkylidene)-2*H*-pyran-2,4(3*H*)-diones as New Photosynthetic Electron Transport Inhibitors

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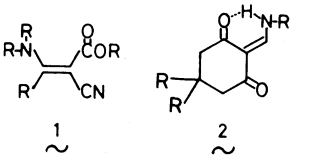
A series of 3-(1-alkylaminoalkylidene)-6-methyl-2*H*-pyran-2,4(3*H*)-diones was newly synthesized, and they were assayed as photosynthetic electron transport (PET) inhibitors because of their structural resemblance to cyanoacrylates and 2-alkylaminoalkylidene-1,3-cyclohexanedione derivatives, which are potent PET inhibitors. Some of the compounds synthesized here showed very high PET inhibition.

The major mode of action for most herbicides is inhibition of photosynthetic electron transport (PET) at the protein-binding quinones, *viz.* between Q_A and Q_B in Photosystem II (PS II).[1] Although the structure–activity relationships of some herbicides for PET inhibition have been reported,[2,3] there is still little information about their binding mode at molecular levels. Advanced molecular biology on the PET system has been unveiling the herbicides binding polypeptide "d-1 (32 kD) protein," including its amino acid sequence.[4~7] A hypothetical folding manner for the peptide chain of "d-1 protein" has been proposed[8] by simulation of the three-dimensional structure of corresponding bacterial "L protein," which was elucidated by an X-ray analysis on the photosystem of *Rhodopseudomonas viridis*.[9] Although the hypothetical "d-1 protein" model suggested a binding niche for herbicides, the actual interaction between herbicides and the niche has remained unknown. Thus, PET research now requires new types of inhibitors with a structure–activity correlation to accumulate more information about the interaction between inhibitors and their binding sites.

Most PET inhibitors such as triazines, ureas, triazinones and anilides interfere with the electron flow at the common site between Q_A and Q_B, due to their binding to "d-1 protein" of PS II.[10] Such inhibitors belong to quite different chemical classes; nevertheless, they are able to displace each other at the binding site in the tylakoid membrane.[11] This fact led us to the concept of overlapping binding sites for these inhibitors.[12]



1 2

Recently, aminocyanoacrylates (**1**)[13,14] and 2-(1-alkylaminoalkylidene)-1,3-cyclohexanediones (**2**, AC)[15] have been reported as new chemical classes in PET inhibitors that interact with the "d-1 protein." Their structural features are quite different from other PET inhibitors, *e.g.*, they commonly possess a carbonyl-conjugated enamine system attached with a lipophilic side chain. Thus, we paid attention to the conjugated enamino part of **1** and **2**, and thought that this structure would be essential for PET inhibition. Herein, we report 2-(1-alkylaminoalkylidene)-1,3-dicarbonyl compounds as

new PET inhibitors.

Synthesis of 3-(1-alkylaminoalkylidene)-2H-pyran-2,4(3H)-diones (AP)

We found that an aminomethylenation reagent consisting of triethyl orthoformate and amines reacted easily with the 3-position of 2*H*-pyran-2,4(3*H*)-diones as shown in Fig. 1.[16] ^{1}H-NMR analysis of the product (**3**) revealed that the vinylic proton was coupled with the amino proton as in the case of **2**, and it was also indicated that two isomeric forms (**3a** and **3b**) existed in about a 4/1 ratio.

Modification of the methylidene in **3** was accomplished by condensing the 3-acyl-6-methyl-2*H*-pyran-2,4(3*H*)-diones with amines to form the aminoalkylidene derivatives. Ethylidene derivatives (**5**) were easily obtained by condensation between dehydroacetic acid and amines in dichloromethane at room temperature for 10 min as shown in Fgi. 1. The structure of **5** was evident by the formation of 4-pyridone derivatives (**7**) from **5** or **6** using a reaction with ammonia in a sealed tube.[17] The pyridone structure **7** was generated by hydrolysis of the pyridone ring and subsequent decarboxylation. Other non-commercial 3-acyl-2*H*-pyran-2,4(3*H*)-diones were prepared by the migration reaction of 4-acyloxy-2-pyrones,[18] and by subsequent condensation with amines to give alkylidene derivatives as shown in Fig. 2. In this report, only 3-propionyl-2*H*-pyran-2,4-(3*H*)-dione was prepared. The ^{13}C-NMR, IR and UV analyses and ferric chloride coloration of these products suggested that alkylaminoalkylidene derivatives preferred the keto form rather than the enol form as in the case of **2**.

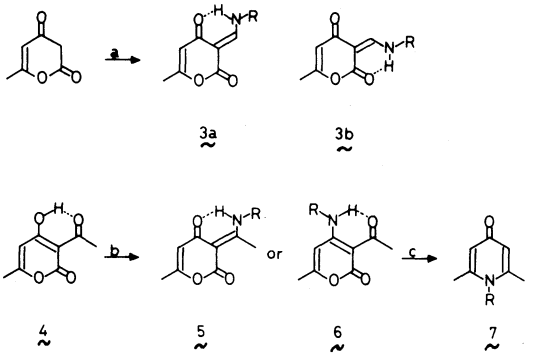


FIG. 1. General Synthetic Route for 3-(1-Alkylaminoalkylidene)-2*H*-pyran-2,4(3*H*)-dione Analogues.
a) $CH(OEt)_3$, RNH_2; b) RNH_2; c) NH_4OH.

Synthesis of other cyclic conjugated enamino compounds

Aminomethylenation at the methylene group of *N*,*N*-dimethylbarbituric acid (**8**) was carried out by a condensation reaction using formamide derivatives under drastic heating.[19] On the other hand, Meldrum's acid (**10**) was aminomethylenated by the orthoformate method as well as the 2*H*-pyran-2,4(3*H*)-diones. In the ^{1}H-NMR spectra of both **9** and **11**, doublets due to the vinylic protons coupling with the amino protons were observed to rationalize the conjugated enamine structures for both series.

PET inhibition

In Table I, the PET inhibitory activities of synthesized compounds possessing a 2-(1-alkylaminoalkylidene)-1,3-dicarbonyl moiety are listed, the *N*-substituents of these compounds being chosen according to the previous data.[13~15] There are some potent PET inhibitors among the synthesized AP series containing ethylidene and propylidene structures, whereas all methylidene derivatives have no activity. This point is one of the most remarkable structural differences between AP to AC. Also, *N*-substituents of AP require lipophi-

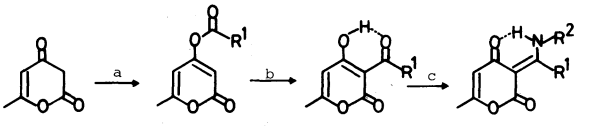


FIG. 2. Modification of the Alkylidene Part of the AP Series.
a) RCOCl, pyridine; b) $AlCl_3$; c) RNH_2.

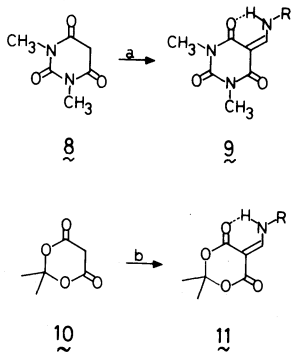
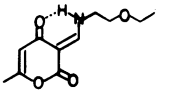
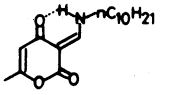
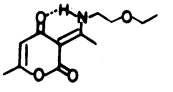
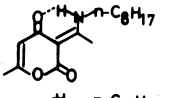
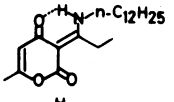
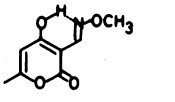
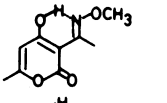
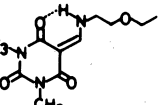
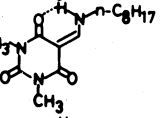
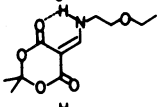
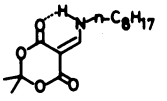


FIG. 3. General Synthetic Route for Other Heterocyclic Analogues.

a) RNHCHO, drastic heating at 250°C; b) $CH(OEt_3)$, RNH_2.

licity to show high PET inhibitory activity in contrast with those of AC. That is to say, it is necessary for the active AC to carry a polar group as an *N*-substituent and a lipophilic group as a substituent on a cyclohexane ring.[15] On the contrary, the active AP carries a lipophilic *N*-substituent and a relatively polar nucleus. These structural differences between AC and AP may cause the change of their binding mode at inhibition sites. In tests, the thermoluminescence experiment using chloroplasts revealed that AP should have a quite different manner from that of other PET inhibitors, and suggested that AP might block the site between Q_B and the PQ pool in the "d-1 protein."[20] Furthermore, only the 2*H*-pyran-2,4(3*H*)-dione ring system was shown to be an active heterocycle among the three types tested here. This fact indicates some validity in the molecular design being considered by only the essentially demanded functionality, the carbonyl conjugated enamine moiety, for highly active PET inhibitors. We are convinced that structure/activity studies on the action of these compounds in a PET system will give much information about a niche for inhibitor binding in the "d-1 protein" because of their strict requirements for this structure. Thus, we concentrated into these studies necessary to make a report on the physiological meaning of the AP series.

TABLE I. PET INHIBITORY ACTIVITY OF 1,3-DICARBONYL DERIVATIVES

No.	Structure	pI_{50}
12		<4
13		<4
14		<4
15		5.2
16		7.5
17		<4
18		<4
19		<4
20		<4
21		<4
22		<4
	DCMU	7.3

EXPERIMENTAL

Hill reaction assay. Spinach (*Spinacia oleracea*) chloroplasts were obtained in the usual way[21] and were stored at −80°C. Photosynthetic activity was measured at pH 7.0 in 2 ml of the medium (50 mM HEPES, 10 mM NaCl, 20 mM methylamine, 50 μM DCIP and 0.5 μg/ml chlorophyll). The

photoreduction of DCIP was measured at 600 nm, and the buffer for chlorophyl dilution was 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$ and 40 mM tricine (pH 7.8). The PET inhibitory activity of the compounds is expressed by pI_{50} values (Table I). These values indicate the negative logarithm of the concentration (M) of those compounds for 50% inhibition of PET.

Chemicals. Melting points were not corrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with JEOL MH-100 (100 MHz) and FX-100 (25.5 MHz) spectrometers, respectively, using tetramethylsilane as an internal standard in $CDCl_3$. IR spectra were recorded with a JASCO A-400 spectrometer, and UV spectra were measured on a Hitachi Model 200-20 spectrometer at a concentration of 1.0×10^{-4} M. High resolution mass spectra were measured with a JEOL DX 303 spectrometer.

1. General synthetic procedure for 3-alkylaminomethylidene-6-methyl-2H-pyran-2,4(3H)-diones (**3**). A mixture of 4-hydroxy-6-methyl-2-pyrone (10 mmol) and triethyl orthoformate (20 ml) was mildly heated at 60 ~ 70°C until the 4-hydroxy-6-methyl-2-pyrone had completely reacted to form triethyl orthoformate (this reaction being checked by TLC). An amine (11 ml) in triethyl orthoformate (10 ml) was then added to the mixture. Triethyl orthoformate was evaporated under reduced pressure and the residue was purified with a silica gel column (100% chloroform) to give **3** almost quantitatively.

2. General synthetic procedure of 3-(1-alkylaminoethylidene)-6-methyl-2H-pyran-2,4(3H)-diones (**5**). Dehydroacetic acid (**4**, 10 mmol) was dissolved in dichloromethane (25 ml), and then a slight excess (11 mmol) of alkylamine in dichloromethane (10 ml) was added. The mixture was stirred for 30 min at room temperature. The solvent was evaporated under reduced pressure and the residue was purified on a silica gel column (hexane : ethyl acetate = 1 : 1) to give **5** in a good yield.

3. General synthetic procedure for 4-acyloxy-6-methyl-2-pyrones. The acyl chloride (11 mmol) was added to a stirred solution of the 6-methyl-2*H*-pyran-2,4(3*H*)-dione (10 mmol) and pyridine (10 mmol) in dry chloroform (50 ml). The mixture was stirred at room temperature for 1 hr, washed with water, and then with diluted hydrochloric acid and saturated aqueous sodium hydrogen carbonate. The organic phase was dried with anhydrous Na_2SO_4 and evaporated to dryness *in vacuo* to give the pure enol ester, which was submitted to the isomerization reaction without further purification.

4. General synthetic procedure for 3-acyl-6-methyl-2H-pyran-2,4(3H)-diones. The enol ester (10 mmol) was added to a stirred suspension of anhydrous aluminum chloride (20 mmol) in dry 1,2-dichloroethane (50 ml). The mixture was stirred for 1 hr at room temperature at 0°C to −10°C, and then poured into a mixture of ice (20 g) and conc. hydrochloric acid (20 g). The organic phase was separated and the aqueous phase was extracted with chloroform. The combined organic phase was washed with water, dried with anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was dissolved in a minimum quantity of ether and treated with 1-normal sodium hydroxide. The aqueous layer contained the sodium salt of 3-acyl-6-methyl-2*H*-pyran-2,4(3*H*)-diones, which was acidified with conc. hydrochloric acid and extracted with ether. The ethereal solution was dried with anhydrous sodium sulfate, concentrated *in vacuo*, and the residue was recrystallized from hexane.

5. General synthetic procedure for 3-(1-alkylaminoalkylidene)-6-methyl-2H-pyran-2,4(3H)-diones. 3-Acyl-6-methyl-2*H*-pyran-2,4(3*H*)-dione (10 mmol) was dissolved in dichloromethane (25 ml), before a slight excess of alkylamine in dichloromethane (10 ml) was added. The mixture was stirred for 30 min at room temperature. The solvent was evaporated under reduced pressure and the residue was purified on a silica gel column (hexane : ethyl acetate = 1 : 1) to give 3-(1-alkylaminoalkylidene)-6-methyl-2*H*-pyran-2,4(3*H*)-diones in a good yield.

6. General synthetic procedure for 5-alkylaminomethylidene-N,N-dimethylbarbituric acids (**9**). A mixture of *N,N*-dimethylbarbituric acid (**8**, 10 mmol) and alkylformamide (15 mmol) was stirred and heated at about 250°C with no solvent for 1 hr, this reaction being checked by gas chromatography. The mixture was poured into water and then extracted with dichloromethane. The organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was finally recrystallized from ethyl acetate and hexane to give **9** in a good yield.

7. General synthetic procedure for 5-alkylaminomethylidene-2,2-dimethyl-1,3-dioxane-4,6-diones (**11**). A mixture of Meldrum's acid (**10**, 10 mmol) and triethyl orthoformate (20 ml) was mildly heated at 60 ~ 70°C until **10** had been completely converted to triethyl orthoformate, this reaction being checked by TLC. Then, an amine (11 mmol) in triethyl orthoformate (10 ml) was added to the mixture and the heating was stopped. The mixture was stirred for 10 min, the triethyl orthoformate was evaporated under reduced pressure, and the residue was purified on a silica gel column (100% chloroform) to give **11** in a good yield.

Chemical data.

6-Methyl-3-propionyl-2H-pyran-2,4(3H)-dione. mp 104 ~ 105°C; IR ν_{max} cm^{-1} (Nujol): 3400 (w), 1720 (s), 1640 (s), 1600 (s), 1545 (s), 1300 (m), 1230 (m), 1000 (s); ^{1}H-NMR δ: 1.15 (CH_3, t, J = 7 Hz), 2.27 (CH_3, s), 3.10 (CH_2, q, J = 7 Hz), 5.94 (CH, s), 16.70 (OH, s); ^{13}C-NMR δ: 8.3, 21.2, 35.8, 102.0, 161.6, 169.3, 181.6, 208.8. *Anal.* Found: C, 59.38; H, 5.55. Calcd. for $C_9H_{10}O_4$: C, 59.33;

H, 5.53.

4-Hydroxy-3-methoxyiminomethyl-6-methyl-2-pyrone (**17**). Oil; IR ν_{max} cm^{-1} (film): 3400 (w), 1725 (s), 1700 (s), 1640 (s), 1595 (s), 1560 (s), 1450 (s), 1300 (s), 1055 (s), 1030 (s); UV λ_{max} (MeOH) nm (ε): 245 (5900), 319 (12600); ^{1}H-NMR δ: 2.24 (CH_3, s), 3.90 (CH_3, s), 5.90 (CH, s), 8.26 (CH, s), 11.40~12.40 (OH, br.); ^{13}C-NMR δ: 20.3, 62.8, 94.4, 100.9, 147.7, 162.7, 164.6, 170.7.

4-Hydroxy-3-(1-methoxyiminoethyl)-6-methyl-2-pyrone (**18**). Oil; IR ν_{max} cm^{-1} (film): 3400 (m), 1720 (s), 1700 (s), 1645 (s), 1595 (s), 1560 (s), 1445 (s), 1410 (s), 1375 (s), 1055 (s); UV λ_{max} (MeOH) nm (ε): 230 (5980), 311 (8240); ^{1}H-NMR δ: 2.36 (CH_3, s), 2.58 (CH_3, s), 4.16 (CH_3, s), 6.20 (CH, s), 14.70 (OH, br.); ^{13}C-NMR δ: 14.1, 20.1, 62.5, 95.7, 101.5, 160.2, 162.4, 163.6, 172.4.

3-(2-Ethoxyethylamino)methylidene-6-methyl-2H-pyran-2,4(3H)-dione (**12**). mp 70~71°C; IR ν_{max} cm^{-1} (nujol): 3400 (m), 3150 (m), 1700 (s), 1650 (s), 1620 (s), 1440 (s), 1315 (s), 1120 (s), 1040 (s); UV λ_{max} (MeOH) nm (ε): 235 (12600), 315 (18200); ^{1}H-NMR δ: 1.20 (CH_3, t, J=7 Hz), 2.15 (CH_3, s), 3.66 (CH_2, q, J=7 Hz), 3.60~3.90 (2×CH_2, m), 5.71 (CH, s), 8.35 and 8.52 (CH, d, J=14 Hz), 11.40~12.50 (NH, br.); ^{13}C-NMR δ: 15.0, 20.1, 50.4, 66.7, 68.5, 96.5, 107.5, 160.9, 162.3, 164.3 and small signals above 90 ppm (96.8, 108.2, 163.8, 164.7, 165.7, 180.7). HRMS. Found: 225.1042. Calcd. for $C_{11}H_{15}NO_4$: 225.1001.

3-Decylaminomethylidene-6-methyl-2H-pyran-2,4(3H)-dione (**13**). mp 88~89°C; IR ν_{max} cm^{-1} (nujol): 3150 (m), 1710 (s), 1660 (s), 1630 (s), 1575 (s), 1310 (s); ^{1}H-NMR δ: 0.88 (CH_3, br.), 1.15~2.00 (6×CH_2, m), 2.14 (CH_3, s), 3.50 (CH_2, t, J=7 Hz), 5.70 (CH, s), 8.27+8.42 (CH, d, J=14 Hz), 11.40~12.00 (NH, br.). *Anal.* Found: C, 69.79; H, 9.28; N, 4.61. Calcd. for $C_{17}H_{27}NO_3$: C, 69.62; H, 9.22; N, 4.78.

3-[1-(2-Ethoxyethylamino)ethylidene]-6-methyl-2H-pyran-2,4(3H)-dione (**14**). mp 42~43°C; IR ν_{max} cm^{-1} (nujol): 3370 (w), 3060 (m), 1690 (s), 1655 (s), 1590 (s), 1355 (s), 1320 (s), 1110 (s); UV λ_{max} (MeOH) nm (ε): 234 (14300), 309 (18200); ^{1}H-NMR δ: 1.18 (CH_3, t, J=7 Hz), 2.08 (CH_3, s), 2.61 (CH_3, s), 3.30~3.70 (CH_2, q, J=7 Hz; 2×CH_2, m), 5.60 (CH, s); ^{13}C-NMR δ: 15.2, 18.3, 19.7, 44.3, 66.9, 67.9, 96.5, 107.6, 162.3, 176.2, 184.3. *Anal.* Found: C, 60.27; H, 7.16; N, 5.84. Calcd. for $C_{12}H_{17}NO_4$: C, 60.24; H, 7.16; N, 5.85.

6-Methyl-3-(1-octylaminoethylidene)-2H-pyran-2,4(3H)-dione (**15**). mp 98~100°C; IR ν_{max} cm^{-1} (nujol): 3650 (w), 3400 (s), 3150 (m), 1700 (s), 1650 (s), 1560 (s), 1380 (s), 1320 (s), 1050 (s); ^{1}H-NMR δ: 0.88 (CH_3, br.), 1.08~2.00 (6×CH_2, m), 2.10 (CH_3, s), 2.42 (CH_3, s), 3.43 (CH_2, dt, J≒6, 6 Hz), 5.60 (CH, s), 10.80~11.50 (NH, br.); ^{13}C-NMR δ: 13.9, 17.8, 19.5, 22.5, 26.7, 29.0, 31.6, 44.1, 96.4, 107.5, 162.2, 162.4, 175.5, 184.2. HRMS. Found: 279.1831. Calcd. for $C_{16}H_{25}NO_3$: 279.1834.

6-Methyl-3-(1-undecylaminopropylidene)-2H-pyran-2,4(3H)-dione (**16**). Oil; IR ν_{max} cm^{-1} (film): 3600 (w), 1700 (s), 1660 (s), 1575 (s), 1480 (s), 1390 (s), 1335 (s), 1270 (s), 1000 (s); ^{1}H-NMR δ: 0.88 (CH_3, br.), 1.10~1.90 (10×CH_2, m), 2.11 (CH_3, s), 3.11 (CH_2, q, J=7 Hz), 3.51 (CH_2, dt, J=6.6 Hz), 5.66 (CH, s), 13.70~14.80 (NH, br.); ^{13}C-NMR δ: (signals below 40 ppm are omitted) 43.4, 95.0, 107.4, 162.1, 163.1, 179.8, 184.6. *Anal.* Found: C, 72.42; H, 9.98; N, 3.99. Calcd. for $C_{21}H_{35}NO_3$: C, 72.16; H, 10.09; N, 4.01.

3-(2-Ethoxyethylamino)methylidene-N,N-dimethylbarbituric acid (**19**). mp 115~117°C; IR ν_{max} cm^{-1} (nujol): 3250 (m), 3160 (m), 1720 (s), 1650 (s), 1485 (s), 1360 (s), 1120 (s), 1100 (s); ^{1}H-NMR δ: 1.20 (CH_3, t, J=7 Hz), 3.28 (2×CH_3, s), 3.51 (CH_2, q, J=7 Hz), 3.52~3.68 (2×CH_2, m), 8.15 (CH, d, J=14 Hz), 9.88~10.40 (NH, br.); ^{13}C-NMR δ: 15.0, 27.0, 27.7, 50.1, 66.8, 68.7, 90.7, 152.2, 160.1, 162.9, 164.7. HRMS. Found: 255.1238. Calcd. for $C_{11}H_{17}N_3O_4$: 255.1220.

3-Octylaminomethylidene-N,N-dimethylbarbituric acid (**20**). Wax; IR ν_{max} cm^{-1} (nujol): 3250 (m), 3160 (m), 1720 (s), 1650 (s), 1485 (s), 1360 (s); ^{1}H-NMR δ: 0.88 (CH_3, br. t), 1.08~1.90 (8×CH_2, m), 3.28 (2×CH_3, s), 3.44 (CH_2, dt, J=7 Hz), 8.12 (CH, d, J=14 Hz), 9.76~10.40 (NH, br.). *Anal.* Found: C, 70.33; H, 8.50; N, 14.22. Calcd. for $C_{15}H_{25}N_3O_3$: C, 60.99; H, 8.53; N, 14.23.

5-(2-Ethoxyethylamino)methylidene-2,2-dimethyl-1,3-dioxane-4,6-diones (**21**). mp 88~89°C; IR ν_{max} cm^{-1} (nujol): 3260 (m), 1720 (s), 1670 (s), 1620 (s), 1325 (s), 1265 (s), 1200 (s), 1120 (s); ^{1}H-NMR δ: 1.20 (CH_3, t, J=7 Hz), 1.72 (2×CH_3, s), 3.34 (CH_2, q, J=7 Hz), 3.60~3.90 (2×CH_3, m), 8.20 (CH, d, J=14 Hz), 9.40~9.90 (NH, br.). *Anal.* Found: C, 54.64; H, 6.93; N, 5.76. Calcd. for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76.

2,2-Dimethyl-5-octylaminomethylidene-1,3-dioxane-4,6-diones (**22**). mp 85~86°C; IR ν_{max} cm^{-1} (nujol): 3270 (m), 3200 (m), 1720 (s), 1670 (s), 1615 (s), 1445 (s), 1325 (s), 1260 (s), 1200 (s), 1010 (s); ^{1}H-NMR δ: 0.88 (CH_3, br. t), 1.16~1.96 (8×CH_2, m), 1.72 (CH_3, s), 3.48 (CH_2, dt, J=7 Hz), 8.18 (CH, d, J=14 Hz), 9.36~9.88 (NH, br.); ^{13}C-NMR δ: 14.1, 22.6, 26.4, 26.9, 29.1, 29.3, 29.4, 30.3, 31.9, 50.6, 84.2, 104.5, 159.7, 164.0, 165.7. HRMS. Found: 311.2102. Calcd. for $C_{17}H_{29}NO_4$: 311.2096.

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