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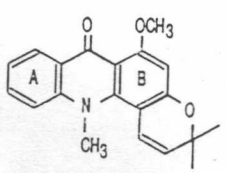
Anticancer Acridone Alkaloids

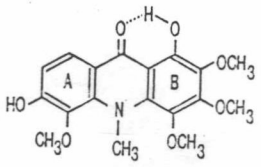
Tsann-Long Su and Kyoichi Aloysius Watanabe

1. INTRODUCTION

Natural products have been used widely for the treatment of cancer. For the past several decades, natural products were studied intensively as source for anticancer chemotherapy. The acridone alkaloid, acronycine (1, Figure 1), which was isolated from *Acronychia baueri* Schott in 1948 (1), was found to have potent antitumor activity (2,3). A variety of naturally-occurring acridone alkaloids are present in the bark and leaves of certain Rutaceae species. Price (4) in 1952 and Saxton (5) in 1972 wrote review articles on acridone alkaloids, including isolation, structure determination and chemical synthesis. Gerzon and Svoboda (6) in 1983 described the detailed antitumor spectrum of acronycine, its mode of action, toxicological studies, and preclinical evaluation. Recently, we have studied structure-activity relationships of a series of naturally occurring acridone alkaloids including 9-acridone and pyranoacridone (acronycine related) derivatives with reference to the inhibition of cell growth and macromolecular biosynthesis of human promyelocytic leukemic cells (7). It was found that multi-substituted (in both A- and B-ring) acridone alkaloids possess significant antitumor activity. Glyfoline (**2**, Figure **2**), a highly oxygenated 9-acridone, was found to be the most potent among the acridone alkaloids tested. It was found to inhibit macromolecular biosynthesis, cell growth of human leukemic HL-60 cell lines in vitro, and growth of several solid tumors in mice. In this article, natural occurrence, chemical synthesis and antitumor evaluation of multi-substituted acridone alkaloids will be discussed.



1 Acronycine



2 Glyfoline

Fig. 1

2. NATURALLY OCCURRING ACRIDONE ALKALOIDS

2.1 Pyrano[2,3-c]acridin-7-one (Acronycine) Derivatives

Table 1 lists derivatives of pyrano[2,3-c]acridin-7-one (acronycine or angular pyranoacridone derivatives) bearing substituent(s) on the A-ring of the molecule which were isolated from Rutaceae plants. Most of the acronycine type derivatives isolated from nature are noracronycines (6-hydroxypyrano[2,3-c]acridin-7-ones). Compounds with substituents at 11-position, such as atalphyllidine [**3**, isolated from Indian plant *Atalantia monophylla* (9)], 11-O-methylatalphillidine [**4**, from *Atalintia ceylanica* (11)] and its N-methyl derivative **5** [baiyumine A, from *Citrus grandis* (12,13)], 11-hydroxy-noracronycine [**6**, from *A. ceylanica* (11)], atalphyllinine [**7**, from *A. monophylla* (9)] and its N-methyl derivative **8** [from *A. ceylanica* (11)] both of which bear a dimethylallyl (prenyl) group at C-5. Noracronycines with substituents at the C-10 and C-11 positions isolated from nature are citracridone I and II [**9** and **10**, respectively, which were isolated from *Citrus depressa* (14,15) indigenous to Taiwan], and 11-hydroxy-10-methoxynoracronycine [**11**, from *Citrus decumna* (16)].

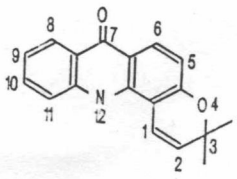
2.2 Pyrano[3,2-b]acridin-6-one (Isoacronycine) Derivatives

In the series of pyrano[3,2-b]acridin-6-ones (isoacronycine or linear pyranoacridone derivatives), only a few compounds were isolated from Rutaceae plants (Table **2**). Pyranofoline [**12**, from *Glycosmis citrifolia* (8)], junosidine (**13**, from *Citrus junos* (17)], glycofoline (**14**, from *G. citrifolia* (8)], and cycloglycofoline [**15**, from *G. citrifolia* (8)] possess an OH or OMe function at the 10 position of the molecule, while honyumine (**16**, from *C. grandis* (18)] has two substituents at C-10 (OH) and C-12 (OMe).

2.3 Acridin-9-one Derivatives

Table **3** lists the natural acridin-9-one (9-acridone) derivatives with multi-substituents on both A- and B-rings of the 9-acridone molecule. Most of these alkaloids are N-methyl-noracridin-9-one (1-hydroxyacridin-9-one) which bear substituents (such as OH, OMe, or prenyl functions) on both A- and B-rings of the molecule. A small group of 9-acridones with substituents at C-1, C-3, and C-5, and at C-1, C-2, C-3, and C-5, of the molecule were found in nature. 1,5-Dihydroxy-3-methoxy-10-methylacridin-9-one (**17**) was isolated from *A. ceylanica* (19). An unusual alkaloid, oligophylidine (**18**), was isolated from *Acronychia oigophylebeia* (20), and its structure was postulated to be 1,8-dihydroxyacridin-9-one on the basis of spectrophotometrical studies. Tecleanthine [**19**, from *Teclea natalensis* (21)], 1,5-dihydroxy-2,3-dimethoxy-10-methylacridin-9-one (**20**) and junosine (**21**) were obtained from *Glycosmis bilocularis* (22) and *C. junos* (13), respectively. A relatively large group of natural 9-acridones containing substituents at C-1, C-3, C-4, and C-5 (**22** - **24**, from citrus plants), C-1, C-3, C-5, and C-6 (**25** - **30**, from citrus plants), C-1, C-2, C-3, C-4, and C-5 [**31** - **34**,

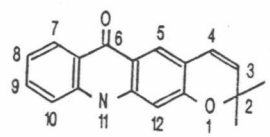
Table 1. The Pyrano[2,-3c]acridin-7-one alkaloids



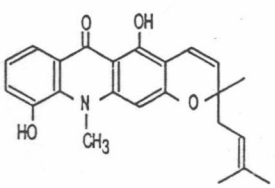
	Compound	5	6	10	11	12	Occurrence	Ref.
1	Acronycine		OMe			Me	A. baueri	1
3	Atalphyllidine		OH		OH	H	A. monophylla	9,10
4	11-O-Methyl-atalphyllidine		OH		OMe	H	A. ceylanica	11
5	Baiyumine A		OH		OMe	Me	C. grandis	12,13
6	11-Hydroxynor-acronycine		OH		OH	Me	A. ceylanica	11,14
7	Atalphyllinine	R	OH		OH	H	A. monophylla	9,10
8	N-methyl-atalphyllinine	R	OH		OH	Me	A. ceylanica	11,10
9	Citracridone I		OH	OH	OMe	Me	C. depressa	14,15
10	Citracridone II		OH	OMe	OMe	Me	C. depressa	14,15
11			OH	OMe	OH	Me	C. decumna	16

R = $-CH_2CH=C(CH_3)_2$

Table 2. The Pyrano[3,2-b]acridin-6-one alkaloids:



	Compound	5	10	11	12	Occurrence	Ref.
12	Pyranofoline	OH	OH	Me		G. citrifolia	8
13	Junosidine	OH	OMe	Me		C. junos	17
14	Glycofoline					G. citrifolia	8
15	Cycloglycofoline					G. citrifolia	8
16	Honyumine	OH	OH	Me	OMe	C. grandis	18



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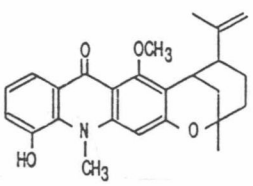

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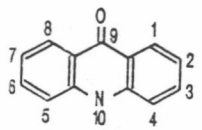
Table 3. The Acridin-9-one alkaloids:



Compound		1	2	3	4	5	6	8	10	Occurrence	Ref.
17		OH		OMe		OH			Me	A. ceylanica	19
18	Oligophylidine	OH		OMe				OH	Me	A. oligphyle	20
19	Tecleanthine	OH	$-O-CH_2-O-$			OMe			Me	T. natalensi	21
20		OH	OMe	OMe		OH			Me	G. biloculari	22
21	Junosine	OH	R	OH		OH			Me	C. juncs	13
22	Citrusinine I	OH		OMe	OMe	OH			Me	C. sinensis	23
23	Citrusinine II	OH		OH	OMe	OH			Me	C. sinensis	23
24	Glycocitrine I	OH		OMe	R	OH			Me	G. citrifoli	8,24
25	Natsucitrine I	OH		OMe		OMe	OH		H	C. natsudaidai	25
26	Natsucitrine II	OH		OMe		OMe	OMe		H	C. natsudaidai	25
27	Grandisine I	OH		OMe		OH	OMe		Me	C. grandis	26
28	Grandisine II	OH		OH		OMe	OH		Me	C. grandis	26
29	Citpressine I	OH		OMe		OMe	OH		Me	C. depressa	14,15
30	Citpressine II	OH		OMe		OMe	OMe		Me	C. grandis	14,26
31	Citbrasine	OH	OMe	OMe	OMe	OH			Me	C. sinensis	23
32		OH	R	OMe	R	OMe			H	A. monophylla	27
33	Atalphylline	OH	R	OH	R	OH			H	A. monophylla	27
34	N-Methyl-atalphylline	OH	R	OH	R	OH			Me	A. monophylla	10,27
35		OH	OMe	OH		OH	OH		Me	C. hybrid	28
36	Atalfoline	OH	OMe	OH		OMe	OMe		Me	C. buxifolia	29
37		OH	OMe	OMe		OMe	OH		Me	P. alatum	30
38	Buntanine	OH	R	OH		OMe	OH		Me	C. grandis	31
39	Prenyl-citpressine	OH		OH	R	OMe	OH		Me	C. grandis	23,31
40	Grandisinine	OH		OMe	R	OMe	OH		Me	C. grandis	26
41	Baiyumine B	OH		OMe	R	OMe	OMe		Me	C. grandis	12
2	Glyfoline	OH	OMe	OMe	OMe	OMe	OH		Me	G. citrifolia	8,24

R = $-CH_2CH=C(CH_3)_2$

from *A. monophylla* (27)], C-1, C-2, C-3, C-5, and C-6 [**35 - 38**, from *Pleiospermium alatum* (30) and citrus plants (31)], C-1, C-3, C-4, C-5, and C-6 [**39 - 41**, from *C. grandis* (12,26)] have been isolated. Glyfoline [**2**, from *G. citrifolia* (8,24)] is the most oxygenated acridone from natural sources .

3. PHYSICAL AND CHEMICAL PROPERTIES

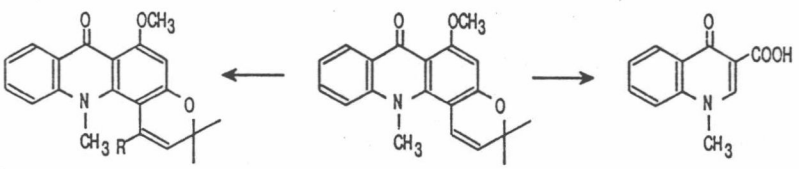
All acridone alkaloids are highly crystalline compounds with yellow to orange color. The acridone alkaloids are very weakly basic and soluble only in strong aqueous mineral acid (e.g. 10% HCl) but not in dilute acid (4). The free alkaloids are easily dissolved in aprotic solvents such as chloroform or ethyl acetate. They are fairly soluble in alcohol, but insoluble in water. The insolubility of acridone alkaloids often causes difficulty in their biological evaluation.

Price (4) noted that the O-methyl function at the peri position of carbonyl group of all acridone alkaloids is not stable in acidic media and is easily de-O-methylated to form the corresponding noracridone (1-OH-acridone) derivatives. For example, treatment of 1-OMe acridone in refluxing alcohol containing conc. HCl gives the corresponding noracridone derivative. The sensitivity of the 1-O-methyl acridone alkaloids to mineral acid raises the question whether the noralkaloids truly exist in nature (4). In noralkaloids, the OH function at the peri position of the carbonyl forms an intramolecular hydrogen bond with the carbonyl group. The formation of the intramolecular hydrogen bond can be detected in the ^{1}H-NMR spectrum at low field ca. δ 12-14.5, or in the IR absorption spectrum at ca. 1600 and 3400 cm^{-1}. Noralkaloids resemble their parent compounds in solubility, and they are also very weakly basic and insoluble in alkali (5). Saxton (5) mentioned that the stability of the intramolecular hydrogen bond of noracridone would be greater when the contribution of the ionic structure such as **42b** is larger (Scheme 1). Therefore, the substituent(s), electron-donating or electron-withdrawing, on the A and B rings of the molecule, may change the strength of the hydrogen bond. Formation of the ionic structure **42b** results in a lower basicity of the noracridone compared with the parent alkaloid (melicopicine, **46**, Figure 4).

42a Scheme 1 42b

Alkylation of the phenolic hydroxy group and the heterocyclic nitrogen in des-N-methyl noracridones is interesting to study. It is known that the phenolic hydroxy at the peri position to the carbonyl is not susceptible to alkylation under ordinary conditions due to the formation of an intramolecular hydrogen bond. O-Alkylation of this hydroxy (1-OH) can be achieved only under forced conditions (NaH/Me_2SO_4) (32), while the other phenolic functions possess normal phenolic properties and, hence, can be O-alkylated easily. For example, 1,3,5-trihydroxyacridin-9-one (**76**) can be selectively benzylated to form the 5-O-benzyl derivative (**77**) (Scheme **9**) upon treatment with benzyl chloride in acetone in the presence of sodium bicarbonate and sodium iodide (33), indicating that the phenolic OH at C-5 is more nucleophilic than those at C-3 and C-1. N-Methylation of the heterocyclic nitrogen can be achieved by prolonged reaction of des-N-methylacridone with K_2CO_3/MeI in refluxing acetone overnight (34), but, a mixture of N-methyl and N,O^1-dimethyl derivatives are usually obtained with the N-methyl product the being predominant one.

Pyranoacridone derivatives contain one reactive double bond in the pyran ring. Acronycine reacts rapidly with bromine in chloroform to give 1-bromoacronycine hydrobromide (**43**, Scheme **2**) (35). When acronycine is treated with nitric acid diluted with ethanol (1:5) (Scheme **2**), 1-nitroacronycine (**44**) is obtained (35), while in warm nitric acid,



43 R = Br
44 R = NO_2

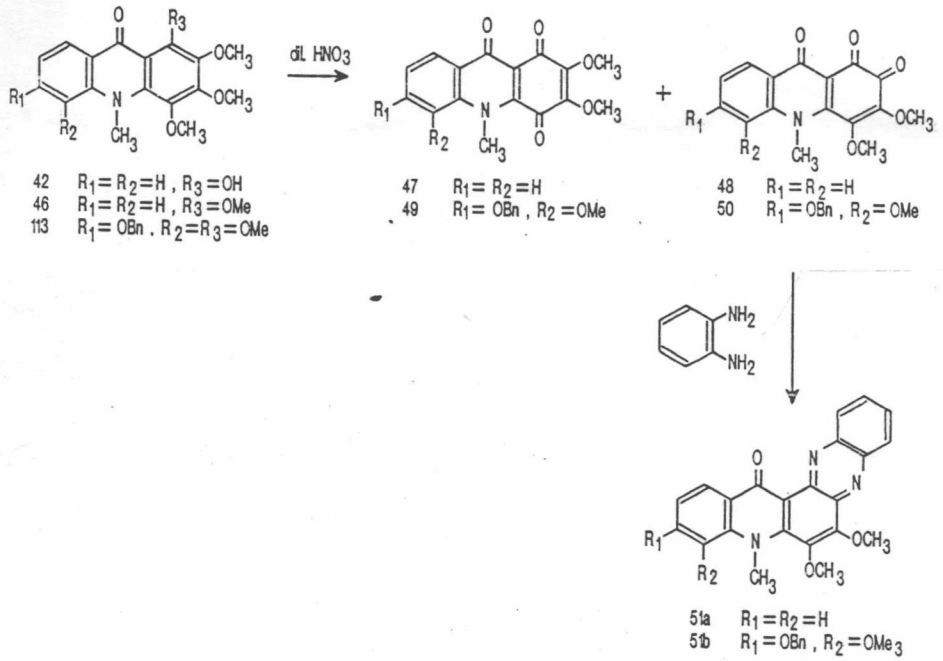
1

45

Scheme 2

acronycine is oxidized to give 1-methyl-4-quinolone-3-carboxylic acid (**45**) (36). Acridin-9-one derivatives, on the other hand, give the quinone derivatives upon oxidation with diluted nitric acid (37,38). Both normelicopicine (**42**) and melicopicine (**46**) on nitric oxidation yield the same mixture of two isomeric quinones, acridin-1,4,9-trione (**47**) and acridin-1,2,9-trione (**48**) (Scheme **3**). During the total synthesis of glyfoline (see below, Scheme **15**), we also found that one of the intermediate, 6-O-benzyl-10-methyl-1,2,3,4,5-tetramethoxy-acridin-9-one (**113**) was also oxidized to form a mixture of the two isomeric quinones (**49** and **50**, Scheme **3**) (39). The mixture can be separated by liquid chromatography, and the 1,2,9-

triones **48** and **50** are readily converted into their corresponding yellow crystalline compounds **51a** and **51b**, by treatment with 1,2-phenylenediamine (37,39).



Scheme 3

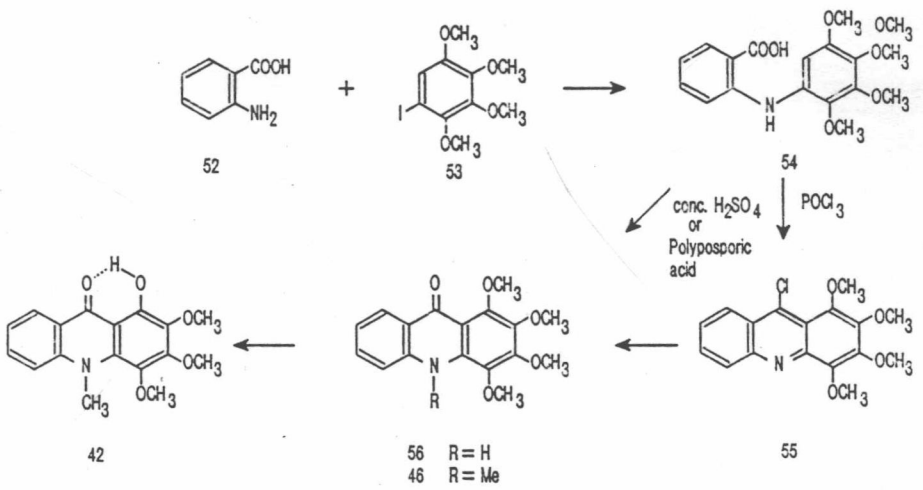
4. CHEMICAL SYNTHESIS OF ACRIDONE ALKALOIDS

Many chemical routes for the preparation of acridin-9-one and pyranoacridine derivatives have been reported. Most of the acridone alkaloids were synthesized via the Jourdan-Ullman condensation. A number of attempts have been made to improve the yield of acridone derivatives. The following are general methods for the synthesis of the alkaloids:

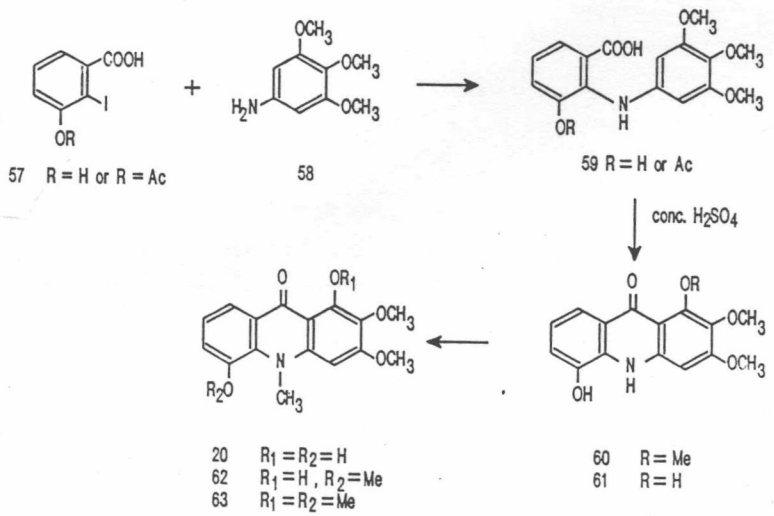
4.1 Jourdan-Ullman Condensation

Ullman reaction was widely used for the synthesis of acridone alkaloids. In 1950 Hughes et al. (40) synthesized melicopicine (**46**) by this procedure as shown in Scheme 4. The synthesis of this alkaloid was achieved by starting from the condensation of anthranilic acid (**52**) with 1-iodo-2,3,4,5-tetramethoxybenzene (**53**). The product, diphenylamine carboxylic acid (**54**), was heated under reflux with phosphorus oxychloride and cyclized to form the 9-chloroacridine derivative (**55**), which was hydrolyzed to give 9-acridone **56**. N-Methylation of **56** by treatment with methyl iodide afforded melicopicine (**46**) in 15%

overall yield. 1,2,3,4-Tetramethoxyacridin-9-one (**56**) can be obtained directly by treatment of **54** with conc. H_2SO_4 or polyphosphoric acid. Upon treatment of **46** with HCl/MeOH, normelicopicine (**42**) was obtained.



Scheme 4

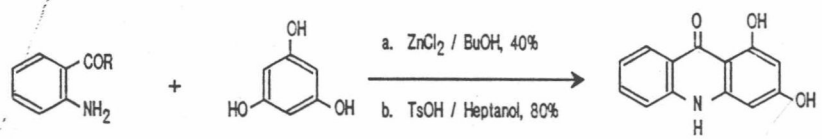


Scheme 5

Bowen *et al.* (41) synthesized 1,5-dihydroxy-2,3-dimethoxy-10-methylacridin-9-one (**20**), which was isolated from *Glycosmis bilocularis* (Rutaceae) by the same authors (Scheme **5**). The total synthesis of **20** was accomplished from diphenylamine carboxylic acid (**59**), which was prepared from by the Jourdan-Ullmann condensation of 3-hydroxy- or 3-acetoxy-2-iodobenzoic acid (**57**, Scheme 5) with 3,4,5-trimethoxyaniline (**58**). Compound **59** was cyclized on warming in conc. sulfuric acid to yield a mixture of **60** and **61**, which were separated by chromatography. Compound **61** was apparently formed by the facile 1-O-demethylation of **60** under the reaction conditions. Methylation of **61** with methyl iodide in the presence of K_2CO_3 in refluxing acetone for four days afforded a mixture of **20**, 1-hydroxy-2,3,5-trimethoxy-10-methylacridin-9-one (**62**), and the fully methylated derivative **63**. The chemically synthesized **20** was identical with the natural product.

4.2 Condensation of Anthranilic Acid with Phloroglucinol:

In 1951 Hughes *et al.* (42) found that reaction of anthranilic acid (**52**) with phloroglucinol (**65**) in refluxing butanol in the presence of zinc chloride formed 1,3-dihydroxyacridin-9-one (**66**) in 40% yield. The synthesis of compound **66** was later improved by Voglet *et al.* (43) by reaction of methyl anthranilate (**64**) with **65** in refluxing heptanol in the presence of Lewis acid (p-toluene-sulfonic acid), which gave **66** in 80% yield (Scheme **6**).



52 R = OH
64 R = OMe

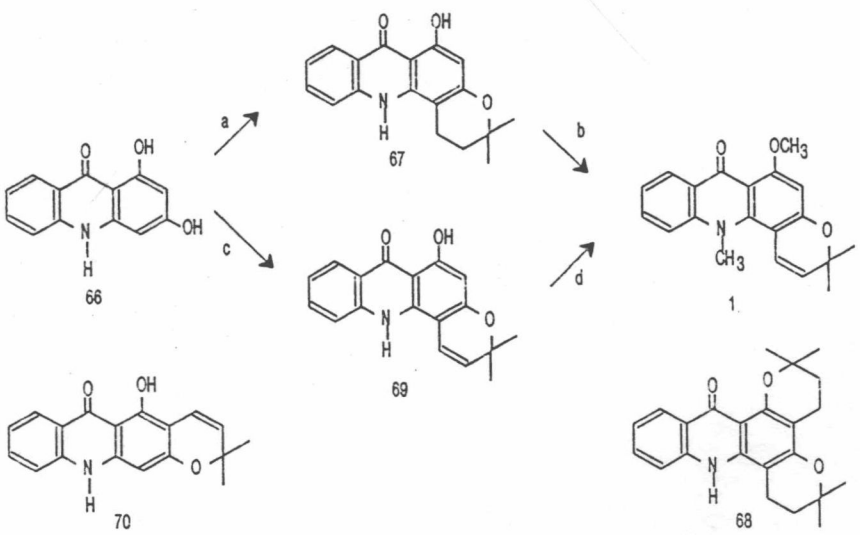
65

66

Scheme 6

Compound **66** was used in many cases as a starting material for the synthesis of acronycine derivatives. Beck *et al.* (32) allowed **66** to react with 1-chloro-3-methyl-2-buten in trifluoroacetic acid with zinc chloride as the catalyst (Scheme 7), and obtained a mixture of 6-hydroxy-3,3-dimethyl-2,3-dihydro-1H-pyrano[2,3-c]-acridin-7-one (**67**) and 3,4,7,8-tetrahydro-2,2,6,6-tetramethyl-2H,6H-dipyrano[2,3-a:2',3'-c]acridin-14(9H)-one (**68**). Compound **67** was then converted into acronycine (**1**) via N-methylation (MeI/K_2CO_3/acetone), dehydrogenation (DDQ/dioxane), and O-methylation (Me_2SO_4/K_2CO_3). The synthesis of acronycine had been improved later by Hlubucek *et al.* (44,45). They treated **66** with 3-chloro-3-methylbut-1-yne in dimethylformamide (DMF) at an elevated temperature (130 °C) in the presence of K_2CO_3 and NaI to obtain des-N-methylnor-

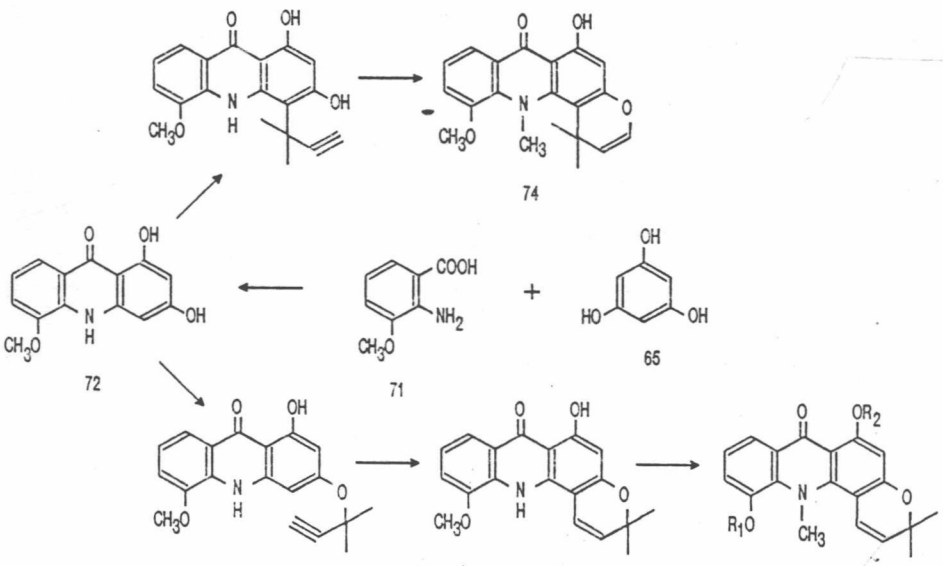
acronycine (**69**) in 85% yield (Scheme 7). Acronycine (**1**) was obtained by treatment of **68** with NaH/Me_2SO_4 in DMF in high yield. No trace of the linear isomer (**70**) was detected by thin layer chromatography in the above cyclization reaction. Later, Fryer *et al.* (46) repeated the same reaction, and found that the linear acronycine (isoacronycine, **70**) was present in a small quantity. They isolated **70** by chromatography using a different solvent system. Almost at the same time, another direct synthesis of acronycine was reported (47). The condensation of 1,3-dihydroxyacridin-9-one with 3-hydroxyisovaleraldehyde dimethyl acetal in the presence pyridine at 150 °C gave a mixture of the angular and linear chromens, **69** and **70**, respectively (Scheme 7).



Scheme 7

By following the procedure developed by Hlubucek *et al.*, but using 3-methoxyanthranilic acid (**71**) as the starting material, 11-hyroxynoracronycine (**6**), which is a constituent of *A. ceylanica* (11) and a metabolite of acronycine in guinea pig (48), was prepared by Adams *et al.* (34) (Scheme 8). Condensation of acridin-9-one **72** with 3-chloro-3-methylbut-1-yne at 70 °C, followed by in situ cyclization of the propargyl ether, however, gave a mixture of 11-O-methylatalphyllidine (**4**) and **74**. The major product **4** had NMR spectral properties

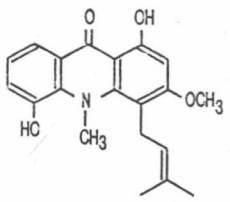
similar to those reported for noracronycine and acronycine. Methylation of **4** with dimethyl sulphate gave the di-O-methyl ether product **73**, which was fused with pyridine/HCl at 200-220 °C to form baiyumine A (**5**). Treatment of **73** with boron tribromide (BBr_3) in methylene chloride at -70 °C, however, afforded 11-hydroxynoracronycine (**6**). Compound **6** was shown to have physical characteristics identical with the alkaloid isolated from *A. ceylanica*. Apparently, de-etherification occurred only for OMe at C-6 and C-11 without change in the pyran ring.



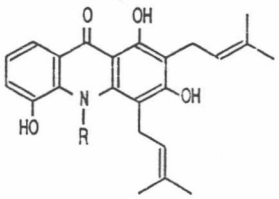
Scheme 8

Using a similar synthetic approach, glycocitrine I (**24**), N-methylatalphylline (**34**), atalphyllidine (**3**), 11-hydroxyacronycine (**86**), and 11-hydroxynoracronycine (**6**) were synthesized by Kapil *et al.* as shown in Scheme **9** (33). 1,3,5-Trihydroxyacridin-9-one (**76**), which was synthesized by the condensation of 3-hydroxyanthranilic acid (**75**) with phloroglucinol (**65**), was selectively O-benzylated to afford 5-benzyloxy-1,3-dihydroxyacridin-9-one (**77**) by treatment with benzyl chloride in the presence of sodium bicarbonate and sodium iodide in refluxing acetone. Prenylation of compound **77** by treatment with 2-methyl-3-buten-2-ol in the presence of boron trifluoride (BF_3) etherate afforded a mixture of the C-4-prenylated acridone **78** and the C-2,4-diprenylated acridone **80**. Compound **78** was N,O-dimethylated to give **79**. Hydrogenolysis of **79** using 10% Pd/C containing sodium ethoxide in absolute ethanol under reflux afforded glycocitrine I (**24**). Benzylation of **80**

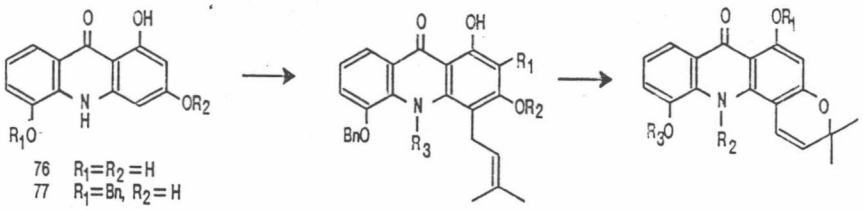
by using the same conditions gave **81**, which was then converted into N-methyl-atalphylline (**34**) via N-methylation (to give **82**) followed by hydrogenolytic debenzylation (10% Pd/C, NaOEt/EtOH). Debenzylation of **82** by catalytic hydrogenolysis afforded the natural product, atalphyllinene (**33**). Condensation of **77** with 3-hydroxyisovaleraldehyde dimethylacetal in pyridine gave angular chromene **83** without isolation of its linear isomer. Atalphyllidine (**3**) was then obtained by debenzylation of **83**. Methylation of **83** by treatment with methyl iodide afforded a mixture of N-methyl **84** and N,O-dimethyl **85** derivatives. Compounds **84** and **85** were separated and debenzylated by hydrogenolysis to give 11-hydroxynoracronycine (**6**) and 11-hydroxyacronycine (**86**), respectively.



24 Glycocitrine-I



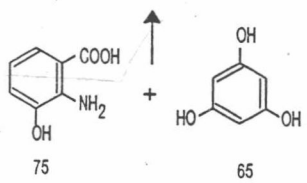
33 R = H Atalphylline
34 R = Me N-Methylatalphyline



76 $R_1=R_2=H$
77 R_1=Bn, $R_2=H$

78 $R_1=R_2=R_3=H$
79 $R_1=H$, $R_2=R_3=Me$
80 R_1 = DMA, $R_2=R_3=H$
81 R_1 = DMA, R_2 = Bn, $R_3=H$
82 R_1 = DMA, R_2 = Bn, R_3 = Me
DMA: dimethylallyl

3 $R_1=R_2=R_3=H$
6 $R_1=R_2=H$, $R_3=Me$
83 $R_1=R_2=H$, R_3 = Bn
84 $R_1=H$, $R_2=Me$, R_3 = Bn
85 $R_1=R_2=Me$, R_3 = Bn
86 $R_1=R_2=Me$, $R_3=H$

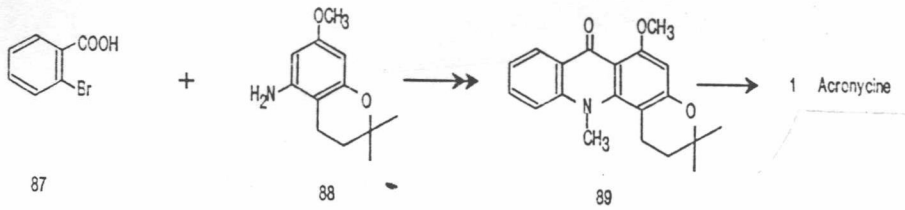


75 65

Scheme 9

It was noted that pyranoacridone arose, probably, from the 3-alkoxy derivative of 1,3-dihydroxyacridin-9-one, which, in many cases, cyclized to form a mixture of angular and linear isomers through Claisen rearrangement (see Scheme 8). Several regioselective syntheses of the angular pyranoacridone (acronycine) have been reported (32,49,50). Beck *et al.* (32) synthesized 5-amino-7-methoxy-2,2-dimethylchroman (**88**), which was then reacted

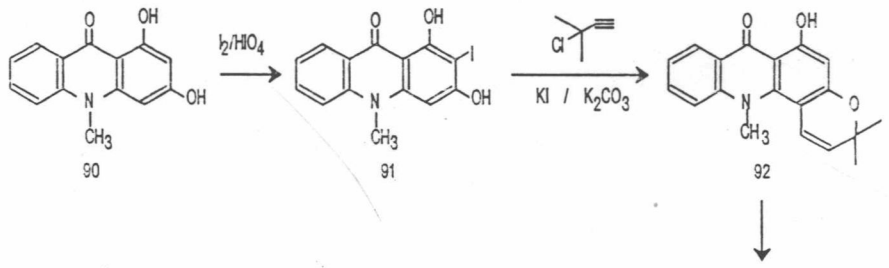
with 2-bromobenzoic acid (87) under the standard Jourdan-Ullman conditions to yield the corresponding diphenylamine carboxylic acid, which was directly converted into 2,3-dihydro-N-methylacronycine (89) by treatment with polyphosphoric acid, followed by N-methylation (Scheme 10). However, dehydrogenation of 89 with DDQ yielded only traces of acronycine (1).



87 88 89

Scheme 10

A simple regiospecific synthesis of acronycine was reported by Reisch *et al.* (49) by the method as shown in Scheme 11. These authors blocked the C-2 of 1,3-dihydroxyacridin-9-one to prevent the linear pyranoacridone isomer formed. Thus, 1,3-dihydroxy-10-methylacridin-9-one (90) was iodinated by treatment with I_2/HIO_4, and the 2-iodo substituted intermediate 91 was then reacted with 3-chloro-3-methylbut-1-yne in DMF in the presence of KI and K_2CO_3 at 100 °C to yield noracronycine (92) in 40% yield. Compound 92 was then converted into acronycine (1).



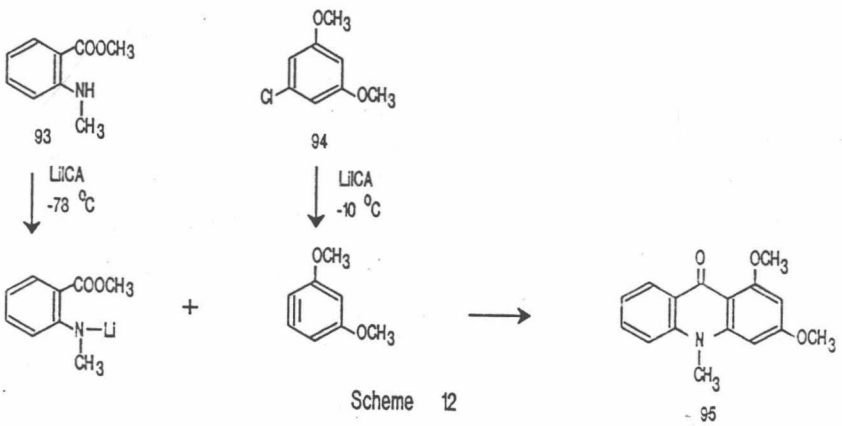
90 91 92

Scheme 11

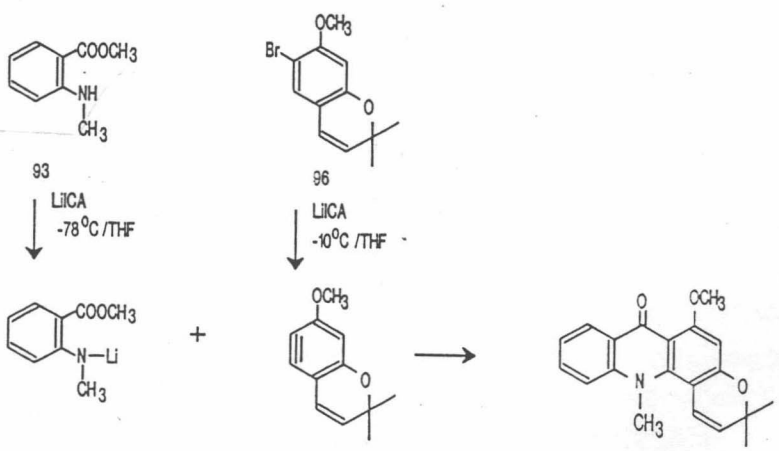
4.3 Condensation of N-Lithio Anthranilates with Benzynes

It was reported (51) that trace amounts of acridones were formed when benzynes were generated by diazotization of anthranilic acids. Thus, traces of undiazotized anthranilic acids react with the benzyne generated from diazotized anthranilic acid resulting in the

formation of acridones. On the basis of this finding, a short synthesis of N-methylacridin-9-one and acronycine was developed by Watanabe *et al.* (52). They found that the lithium salt of methyl N-methylanthranilate (**93**) easily coupled with benzynes giving rise to various N-methylated acridin-9-ones (compound such as **95**) (Scheme **12**). In these reactions, benzynes were generated by treatment of various halobenzenes (such as 1-chloro-3,5-dimethoxybenzene, **94**) with lithium N-isopropylcyclohexylamide (LICA), which was found to be much more effective than lithium diisopropylamide (LDA) and lithium 2,2,6,6-tetramethylcyclohexyl-amide (LTMP). The yields of various acridones synthesized by this method were in a range of 30-68%. By utilizing the above method, a short synthesis of acronycine was developed (Scheme **13**) (52). Thus, reaction of **93** with 6-bromo-7-methoxy-2,2-dimethyl-pyranobenzene (**96**) gave acronycine (**1**) directly in 41% yield, which was



Scheme 12

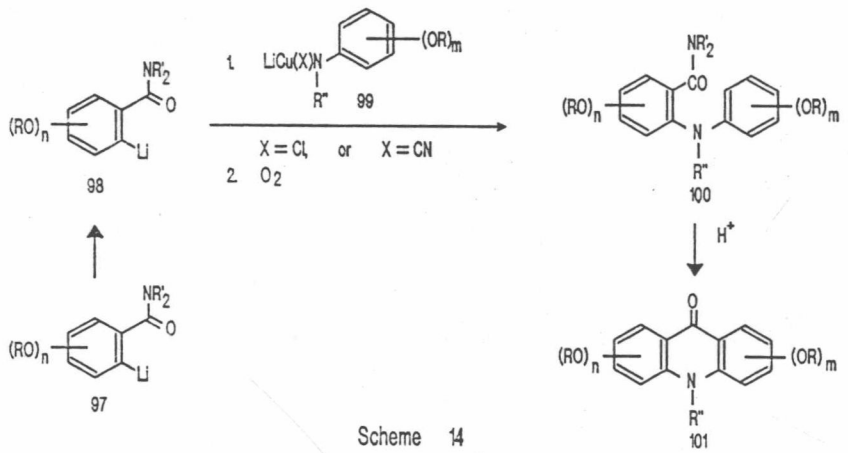


Scheme 13

identical with an authentic sample. This simple synthetic strategy may be applied to the synthesis of other multi-substituted acridin-9-ones and acronycine derivatives.

4.4 Oxidative coupling reaction of ortho-lithiated benzamide with anilido-chloro or -cyano cuprates

A regiospecific introduction of a N-substituent at the ortho position of benzamides has been applied to the synthesis of acridin-9-one derivatives by Snieckus *et al.* (53) (Scheme 14). Lithiation of tertiary benzamides **97** occurred only at the ortho position of the amide function. The resulting product, ortho-lithiated benzamide **98**, was then reacted with the anilido cuprates **99**, which was generated from the lithioanilide and CuCl or CuCN, respectively. The condensation products were oxidized to form N-arylanthranilamide **100** (in 18-63 % yield), which were then directly cyclized into the acridin-9-ones **101** by treatment with trifluoroacetic acid, heptafluorobutyric acid or formic acid under reflux (in 26-95% yield). Various alkoxy-substituted tertiary benzamides underwent coupling reactions with aniline derivatives to form acridin-9-ones.



Scheme 14

5. ISOLATION, STRUCTURE AND CHEMICAL SYNTHESIS OF GLYFOLINE

Glyfoline (**2**), was originally isolated from the root and stem bark of *Glycosmis citrifolia* (Willd.) Lindl. by Wu *et al.* (8,24) in 1982. This plant is a wild shrub indigenous to Taiwan, and has been used as a folk medicine for the treatment itch, scabies, boils, and ulcers. The ethanolic extracts of the root and stem bark of the plant were partitioned between chloroform and water. The chloroform layer was separated, dried, and the residue was extracted with diethyl ether. The ether-soluble fraction was chromatographed over a silica

gel column washing successively with benzene, diisopropyl ether, diethyl ether, and acetone. Glyfoline was isolated from the diisopropyl ether fraction and was eluted with benzene-acetone (19 : 1) as orange colored needles, $C_{18}H_{19}NO_7$, mp 215-217 °C, in 0.0014% yield. Its UV spectrum suggested the presence of a 9-acridone nucleus in the molecule. The IR bands at 1610 and 3400 cm^{-1}, and the ^{1}H-NMR ($CDCl_3$) signals at δ 8.76 and 14.10 (D_2O exchangeable) indicated that the alkaloid contains two phenolic hydroxy functions. The lower signal at δ 14.10 and IR band at 1610 cm^{-1} indicated that one of the phenolic OH forms an intramolecular hydrogen bond with the peri carbonyl function, and is located at C-1 of the 9-acridone nucleus. The aromatic-proton range of the ^{1}H-NMR spectrum showed only two one-proton signals, ortho-coupled to each other, at δ 6.90 and 7.93. The lower signal (δ 7.93) is characteristic for the proton at C-8 of the 9-acridone molecule. The location of the hydroxy group at C-6 (not at C-5) was assigned by an NOE experiment on the methoxymethyl ether derivative of glyfoline. Upon decoupling the methylene protons of the methoxymethoxyl function (δ 5.36), a 12% enhancement of the signal at δ 6.90 (H-7) was observed. The structure of the natural alkaloid was thus elucidated after considering all aspects of its spectral and elemental analyses. Recently, the structure of glyfoline was further confirmed by chemical synthesis in our laboratory as shown in Scheme **15** (39,54). The known compound, 4-benzyloxy-3-methoxy-2-nitrobenzoic acid (**107**) (55), was reduced to the corresponding anthranilic acid (**108**). Compound **107** was synthesized from vanillin (**102**) via O-acetylation (Ac_2O/NaOH), nitration (fuming nitric acid), and hydrolysis (NaOH), followed by O-benzylation (benzyl chloride/NaOH) and oxidation (K_2MnO_4/Me_2CO). Condensation of **108** with 1-iodo-2,3,4,5-tetramethoxybenzene (**53**) gave the 4-benzyloxy-3-methoxy-N-(1,2,3,4-tetramethoxyphenyl)anthranilicacid (**109**) in 60% yield by refluxing in a mixture of Cu, Cu_2O, and potassium carbonate in diglyme. The carboxylic acid **109** was treated with polyphosphoric acid at 80 °C for 2.5 h to afford the de-O-benzylated acridin-9-one (**111**) in 25% yield. The cyclization of **109** was later improved by modification of the procedure developed by Cain *et al.* (56). Compound **109** was converted into the piperidide derivative **110** by treatment with thionyl chloride in pyridine, followed by treatment with piperidine and triethylamine. The piperidide **110** was then treated with phosphorus oxychloride, and the product was hydrolyzed to give the desired O-benzylated 9-acridone **112** in 45% yield. Compound **112** was N-mehtylated to give **113** by treatment with methyl iodide in refluxing acetone in the presence of potassium carbonate. The methoxy function at C-1 of **113** was selectively hydrolyzed with conc. hydrochloric acid in methanol under reflux to yield 1-hydroxyacridin-9-one **114**, which was then de-O-benzylated by catalytic hydrogenolysis to give glyfoline (**2**). The chemically synthesized glyfoline is