POLYMERIZATION AND/OR REARRANGEMENT REACTIONS OF ACRONYCINE AND RELATED COMPOUNDS†

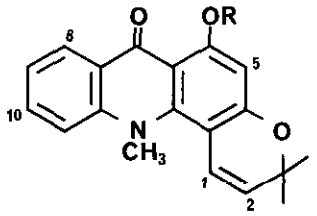
Shinji Funayama†† and Geoffrey A. Cordell*

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, The University of Illinois at Chicago, Chicago, Illinois 60612, USA

Abstract - By treating acronycine or noracronycine with methanolic hydrochloric acid, several oligomers were obtained, including compounds possessing a rearranged partial structure. We have also succeeded in the selective syntheses of monomers and/or oligomers of noracronycine and related compounds by altering the reaction condition(s). Further, polymerization and/or rearrangement reaction mechanisms were examined by the combination of the reactions using D-substituted reagents and ^{1}H-nmr spectroscopic analysis.

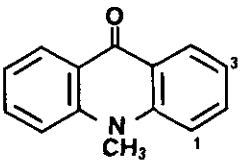
1. INTRODUCTION

Acronycine (1), isolated from the bark of the Australian scrub ash Baurella simplicifolia (Endl.) Hartley (Rutaceae) (syn. Acronychia baueri Scott) in 1948 was one of the first acridone alkaloids to be isolated from natural sources[1-3]. Subsequently, it was shown that 1 possessed the broadest antitumor spectrum of any known alkaloid[4-6].



1 R = CH_3

6 R = H



2

The chemical structure of acronycine (1) was finally established in 1966. Until that time it had been uncertain whether the prenyl moiety attached to the C_2-O position of the acridone (2) skeleton was cyclized to C_1, as in structure 1 (angular form), or to C_3 to have structure 3 (linear form)[7-9]. Through

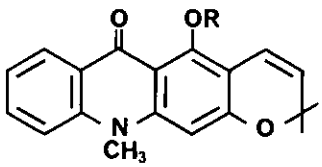
† Dedicated to the memory of Professor Tsunematsu Takemoto.

†† Present Address: The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

a combination of chemical degradation[10], spectroscopic[11,12], crystallographic[13] and synthetic[14] studies the structure of acronycine (1) was established as the angular form and compound 3, which possesses the linear arrangement became known as isoacronycine.

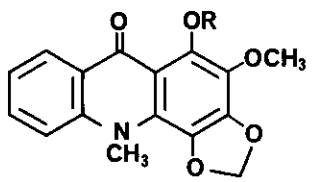
Though about fifty naturally occurring acridone alkaloids have been reported, all of them from rutaceous plants, very little is known about the chemical characteristics of this group of compounds.

It was reported that when melicopine (4) was refluxed in a mixture of 10N HCl-EtOH (1:2.5), normelicopine (5) was obtained in a very good yield[15]. On the other hand, Brown et al. reported that when acronycine (1) was treated under the same reaction conditions, noracronycine (6) could not be isolated from the reaction mixture.[7]



3 R = CH_3

8 R = H



4 R = CH_3

5 R = H

As a part of a larger program examining the potential development of derivatives of acronycine as antitumor agents, we were interested in these reports and these experiments were reexamined. As a result, it was shown that the reaction product was a complex mixture of more than ten compounds including noracronycine (6).

Through the isolation and characterization of these reaction products it became apparent that not only demethylation, but also rearrangement and/or polymerization reactions, had occurred and that dimers, trimers, tetramers and pentamers had been synthesized[17,18,25,27]. These were the first reports of such intermolecular condensation reactions via a C-C bond in the acridone alkaloids series.

On the other hand, selective syntheses and studies of the mechanisms of formation of various dimers, trimers and tetramers have been established[17,20,23,24,25]. In addition, spectroscopic studies of acronycine (1) and related compounds [16], rearrangement of angular type acridone derivatives to linear type compounds and their reaction mechanisms[21,22,24,26] as well as phytochemical studies on Baurella simplicifolia[19] have been conducted.

In this review article, the chemical and spectroscopic characteristics of the reaction products are discussed, together with the selective syntheses of the various polymers of acronycine and related compounds and studies on the mechanisms of the polymerization and rearrangement reactions through the combination of deuterium labelling and nmr spectroscopy.

2. REACTIONS OF ACRONYCINE (1) AND RELATED COMPOUNDS WITH HOT METHANOLIC HCL

2.1. Reaction of Acronycine (1) with Hot Methanolic HCl

Acronycine (1) was dissolved in methanolic HCl and refluxed for 6 h. The reddish reaction mixture was diluted with water, and work up of the $CHCl_3$ soluble fraction afforded an orange-red powder[17]. Tlc analysis showed the presence not only of noracronycine (6), but also many new products and a considerable amount of starting material.

2.2. Reaction of Noracronycine (6) with Hot Methanolic HCl

When the reaction was repeated with noracronycine (6), the same reaction mixture, except for acronycine (1), was produced in better yield[17]. It was concluded that the new products were derived from 6 which had been generated through demethylation of 1. In subsequent experiments, 6 was used as the starting material. Noracronycine (6) was prepared in 90% yield by treating the HCl salt of 1 (red needles) at 140℃ for 1 hour[16].

2.3. Tlc Analysis of the Reaction Mixture

The compounds appearing when the crude reaction product was developed with benzene-EtOAc (9:1) on silica gel (Fig. 1) were named AB-1, AB-2, AB-3, AB-4, AB-5A, AB-5B, AB-6A and AB-6B, respectively. The name AB derived from the original plant name (Acronychia baueri) from which acronycine (1) had been isolated.

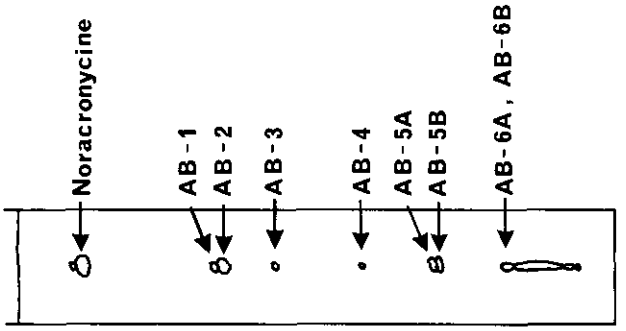


Fig. 1. Tlc of the reaction mixture (Kieselgel 60 F_{254}, Merck, benzene-EtOAc 9:1).

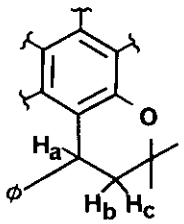
3. PURIFICATION AND CHARACTERIZATION OF THE REACTION PRODUCTS

3.1. Isolation and Characterization of AB-1 and AB-2

The reaction product was chromatographed over silica gel followed by repeated preparative Tlc to afford AB-1 and AB-2 as yellow powders[17].
Uv spectra of these compounds were characteristic of those of acridone alkaloids and a molecular ion at m/z 614 was observed in both compounds, indicating

a mass twice that of 6 (MW 307). On the other hand, the ^{1}H-nmr spectra of these compounds were very similar to each other. Two N-methyl moieties, two hydrogen-bonded phenolic OH groups and two pairs of geminal methyl groups were common in both compounds. Thus, it was concluded that both AB-1 and AB-2 were dimers of noracronycine (6).

In the ^{1}H-nmr spectra of these compounds, signals attributed to three hydrogens which had not been observed in the ^{1}H-nmr spectrum of 6 were observed. Whereas two of the signals assigned to C_1-H, C_2-H and C_5-H of noracronycine (6) disappeared in the ^{1}H-nmr spectra of AB-1 and AB-2. Therefore, it was considered that the C_1 position of one noracronycine (6) unit was attached to the C_5 position of the second 6 unit. The signals attributed to the three hydrogens described above were explained as indicated in Fig. 2[17]. But the structural difference between these two compounds was still unknown.

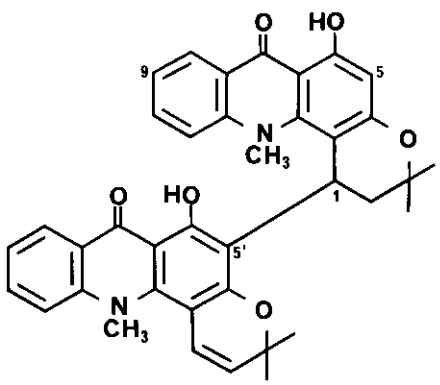


	AB-1	AB-2
H_a	δ 5.165 (1H, dd, J= 7, 12 Hz)	δ 4.904 (1H, dd, J= 8, 12 Hz)
H_b (ax)	1.865 (1H, dd, J= 12, 12 Hz)	2.235 (1H, dd, J= 12, 12 Hz)
H_c (eq)	2.117 (1H, dd, J= 7, 12 Hz)	2.104 (1H, dd, J= 8, 12 Hz)

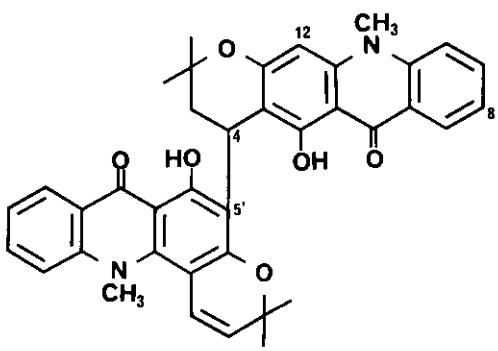
Fig. 2. Newly appeared ^{1}H nmr signals of AB-1 and AB-2.

3.2. Structures of AB-1 (7) and AB-2 (9)

Through the X-ray crystallographic analysis of AB-1 (Fig. 3), the structure of this compound was defined 7, i.e. possessing the angular-angular type (A-A type) skeleton. On the other hand, we were surprised to find that the structure of AB-2 was defined as 9, i.e., a dimer composed of a linear (L type) isonoracronycine (8)



7



9

unit and an angular noracronycine (6) unit to form an L-A skeleton (Fig. 5). Through the X-ray analysis of AB-1 (7), it was shown that an acetone molecule lay between two AB-1 molecules (Fig. 4) and that AB-1 (7) possessed a bent or curled (ca 90°) structure whereas AB-2 (9) possessed a stretched shape (Fig. 6)[17]. Physicochemical properties of these compounds have been defined[23].

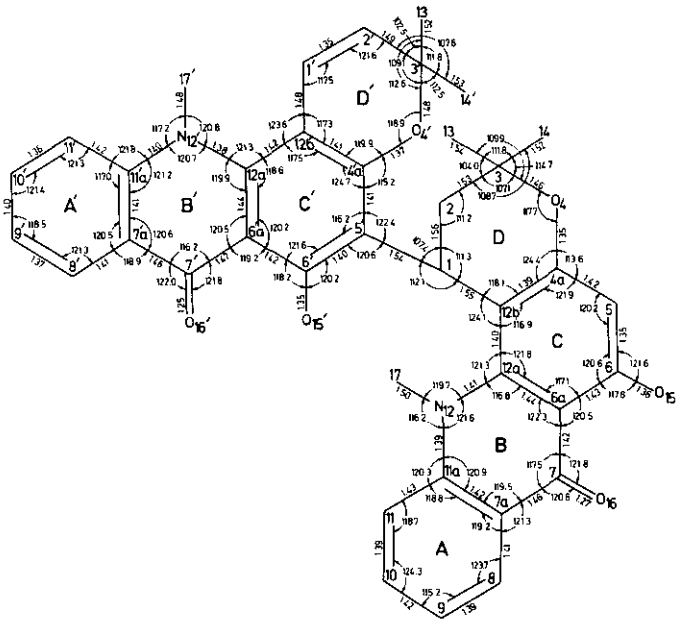


Fig. 3. Atomic distances and angles of AB-1. Standard deviations of atomic distances are less than 0.01 Å, and of the angles less than 0.9°.

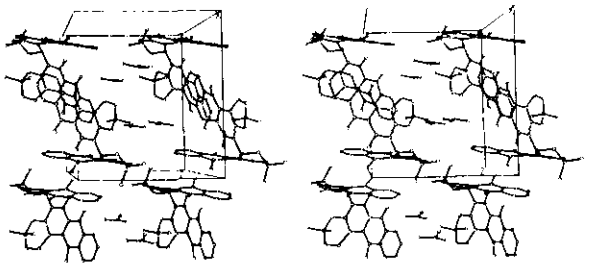


Fig. 4. Crystal structure and molecular packing in the unit cell of AB-1. Acetone molecules are visible in the channel between the two layers of stacking.

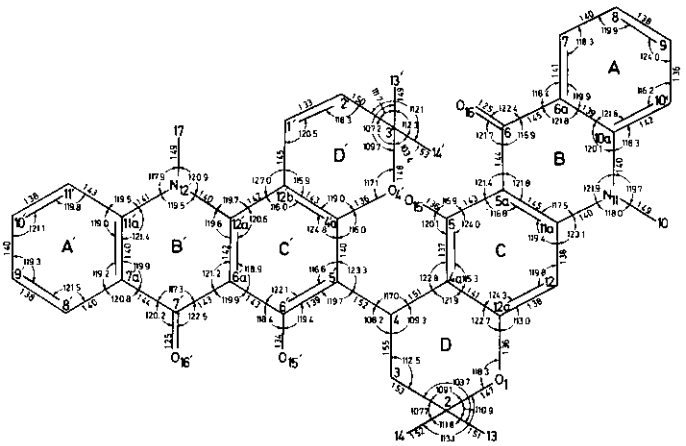


Fig. 5. Atomic distances and angles of AB-2.

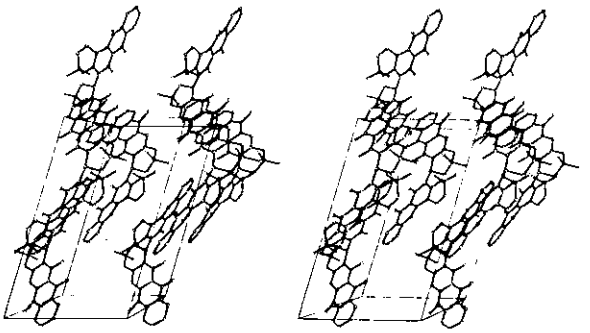
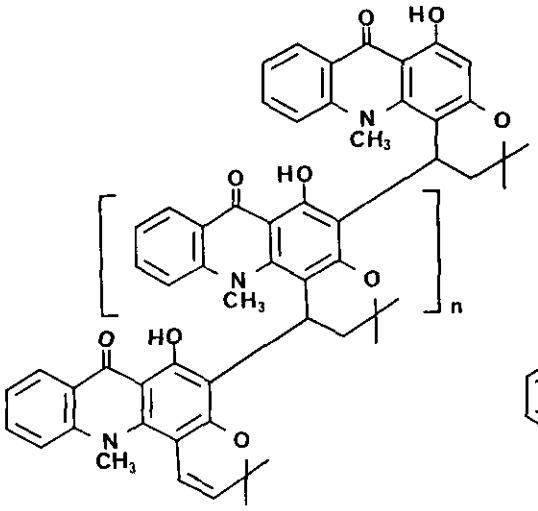
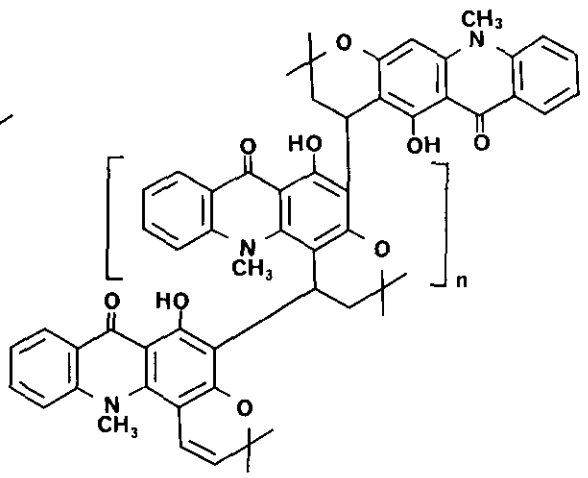


Fig. 6. Crystal structure and molecular packing in the unit cell of AB-2.

3.3. Isolation and Structure Elucidation of AB-3 (10), AB-4 (11), AB-5A (12), AB-5B (13), AB-6A (14) and AB-6B (15)

Because the structures of AB-1 (7) and AB-2 (9) were established and the physicochemical properties of these compounds defined[17,23], structures of AB-3 (10)[18], AB-4 (11)[27], AB-5A (12)[25], AB-5B (13)[26], AB-6A (14)[27] and AB-6B (15)[27] could be established through the physicochemical analysis of these compounds. Namely, structures of these compounds were concluded to be composed of 6 and 8 and possessed the A-A-A (10)[18], A-A-A-A (11)[27], A-A-A-A-A (12)[25], L-A-A-A (13)[26], L-A-A-A-A (14)[27] and A-A-A-A-A (15)[27] skeletons, respectively. Since both AB-5A (12) and AB-6B (15) are pentamers composed of all angular units, it was deduced that there are differences in the three dimensional structures of these compounds.





10 n = 1 **12** n = 3

11 n = 2 **15** n = 3

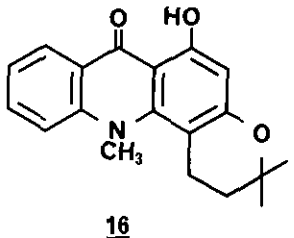
13 n = 2

14 n = 3

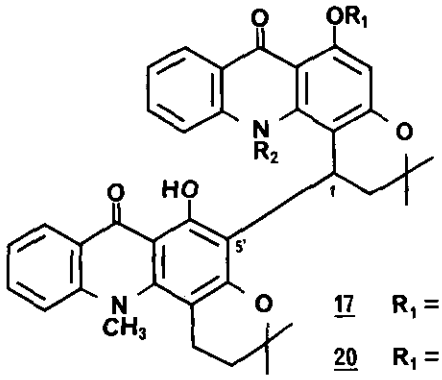
4. SELECTIVE SYNTHESIS OF AB-1 (7), AB-2 (9), AB-3 (10), AB-4 (11) AND RELATED COMPOUNDS

4.1. Selective Synthesis of Various Polymers through Polymerization

Strategically, it was considered that the polymerization reaction could be carried out between a small amount of noracronycine (6) and an excess of dihydronoracronycine (16). Because 16 lacks the C_1-C_2 double bond and polymerization between two molecules of 16 is impossible, it was considered that dihydro AB-1 (17) lacks the coupling site to polymerise with 16 and given the low concentration of 6, the coupling reaction should stop when 6 was thoroughly used. It was initially established that no reaction occurred when dihydronoracronycine (16) was refluxed alone with methanolic HCl[17].



16



17 R_1 = H, R_2 = CH_3

20 R_1 = R_2 = CH_3

21 R_1 = CH_3, R_2 = H

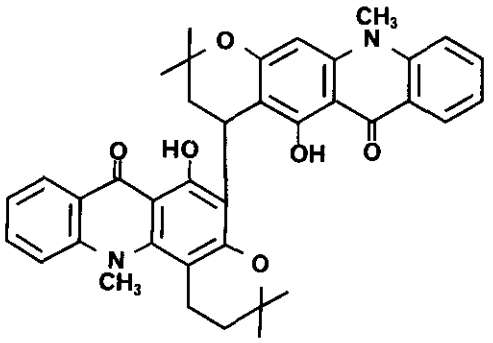
4.1.1. Dimerization Reaction between Noracronycine (6) and Dihydronoracronycine (16)

Noracronycine (6) and dihydronoracronycine (16) were mixed in the ratio 1:10 and refluxed in methanolic HCl for 6h. By mixing 6 and 16 in the ratio 1:10, it was anticipated that a molecule of 6 would react preferentially with a dihydronoracronycine molecule (16) as described above. In the event, a dimer of MW 616 was obtained in 86% yield[17]. Because the product was estimated to be dihydro AB-1 (17), a dihydro derivative of AB-1 was prepared by catalytic hydrogenation of AB-1 (7). Comparison of the two products indicated that the product obtained through the coupling reaction was not identical with dihydro AB-1 (17) prepared by catalytic hydrogenation of 7. Therefore, either the postulated structure of the synthetic dimer and/or the structure of dihydro AB-1 (17) prepared by the hydrogenation of AB-1 (7) was incorrect.

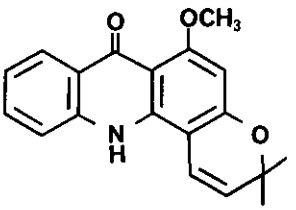
Interestingly, the coupling reaction product was found to be identical with dihydro AB-2 (18) prepared by catalytic hydrogenation of AB-2 (9)[17]. Consequently, in the coupling reaction between 6 and 16, both a coupling reaction and a rearrangement of the angular system of the upper unit to the linear system had occurred.

The facile nature of this rearrangement led us to a reaction in which AB-1 (7) was refluxed in methanolic HCl; the products were the linear-angular dimer AB-2 (9) and noracronycine (6)[17]. By treating AB-2 (9) under the same conditions, noracronycine (6) and isonoracronycine (8) were produced[17]. These experiments suggest that AB-1, possessing the structure 7, is probably an intermediate in the formation of AB-2 (9) and is constructed from two noracronycine units.

Interestingly, when a coupling reaction between 6 and 16 was conducted at room temperature for 3 days, a 1:1 mixture of dihydro AB-1 (7) and dihydro AB-2 (18) was produced[23].



18



19

4.1.2. Coupling Reactions between Acronycine (1) or Des-N-methylacronycine (19) and Dihydronoracronycine (16)

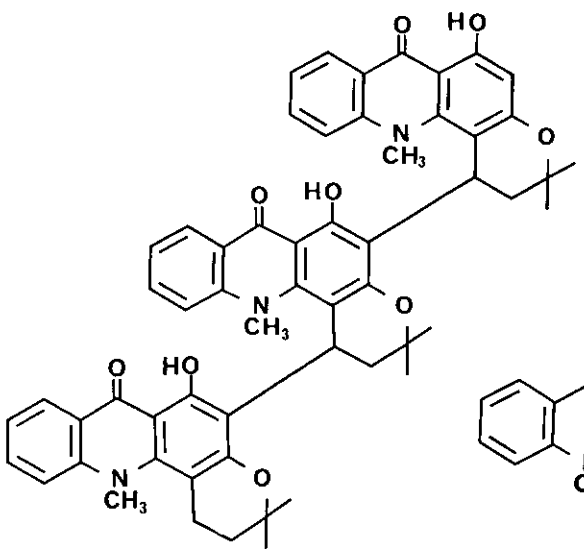
With these resuts in hand, we began to examine further the selective synthesis

of other dimers of noracronycine (6) and related compounds[23]. Thus, when acronycine (1) was used instead of 6, and a 1:10 mixture of 1 and 16 was refluxed in methanolic HCl on a steam bath for 6h, O-methyl dihydro AB-1 (20) was obtained. Significantly, no rearrangement of the upper unit was observed in this coupling reaction. The angular-angular skeleton of this compound was demonstrated through nOe difference experiments. On the other hand, when des-N-methylacronycine (19) was used in place of acronycine (1) in the coupling reaction described above, des-N-methyl-O-methyldihydro AB-1 (21) was obtained. A 6-hydroxy group and a chromene unit must therefore be essential for the angular to linear rearrangement reaction to be observed.

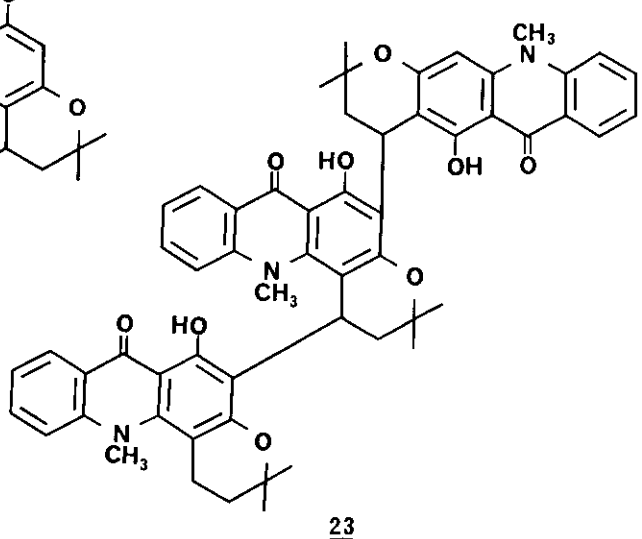
An improved yield of 20 was obtained when a 1:2 mixture of 1 and 16 was treated in the same way. The angular-angular array of this compound was demonstrated when the identical compound was obtained through the coupling of acronycine(1) and dihydronoracronycine (16) in methanolic HCl at room temperature, and through the observation of an upfield shift of a doublet assigned to the proton peri- to the N-methyl group. Such an upfield shift of the signal attributed to the lower unit protons peri- to the N-methyl group was consistently observed in the ^{1}H-nmr spectra of oligomers possessing angular-angular moieties and was useful in elucidating the skeleton of the oligomers.

4.1.3. Coupling Reaction between AB-1 (7) or AB-2 (9) and Dihydronoracronycine (16)

During a study of the trimerization of noracronycine (6), an attempt was made to synthesize dihydro AB-3 (22) in order to demonstrate that AB-3 (10) posssessed the angular-angular-angular structure. The synthesis of 22 was attempted by coupling AB-1 (7) with dihydronoracronycine (16) in methanolic HCl in the same manner as described above (4.1.1, 4.1.2)[20].



22



23

AB-1 (7) and dihydronoracronycine (16) were mixed in the ratio 1:10 and refluxed in methanolic HCl for 6h. By mixing 7 and 16 in this ratio, it was anticipated that a molecule of AB-1 (7) would preferentially react with a dihydronoracronycine (16) molecule. Two reaction products were detected by tlc. One of the two compounds was readily identified as unreacted dihydronoracronycine (16), and it was thought that the other compound was a trimer, namely dihydro AB-3 (22). However, direct comparison of the product with authentic dihydro AB-3 (22), prepared by the catalytic hydrogenation of AB-3 (10), indicated that these compounds were not identical[20].

Consequently, we initially envisaged this new compound to possess the linear-angular-angular structure 23, since it was known that AB-1 (7) could be rearranged to AB-2 (9) under these reaction conditions. In order to confirm this structure, a synthetic route involving the coupling of AB-2 (9) and dihydronoracronycine (16) was studied.

AB-2 (9) and dihydronoracronycine (16) were mixed in the ratio 1:10 and refluxed in methanolic HCl. A compound identical with the product of the coupling of AB-1 (7) and dihydronoracronycine (16) under reflux was isolated, as well as unreacted 16[20].

However, dihydro AB-3 (22) was synthesized by coupling AB-1 (7) and dihydronoracronycine (16) at room temperature for 24h. As expected, no rearrangement occurred under these reaction conditions. From this reaction mixture, in addition to dihydro AB-3 (22) and unreacted dihydronoracronycine (16), a compound with m/z 616 (M^+) was also isolated. Closer examination of this compound indicated that it was a dimer comprised of one molecule of noracronycine (6) and one molecule of dihydronoracronycine (16), namely dihydro AB-1 (17)[20].

We were consequently stimulated to reexamine the nature of the products from the coupling of AB-1 (7) and dihydronoracronycine (16), and of AB-2 (9) and dihydronoracronycine (16) under reflux. In the course of this analysis it was established that the products were identical and exhibited a molecular ion at m/z 616. Clearly, our evaluation that this compound was a trimer possessing the linear-angular-angular system 23 was erroneous. Through direct comparison with an authentic sample, these products were identified as dihydro AB-2 (18)[20].

From these results, it was evident that during the reaction under the reflux, the C_1-C_5· bond of AB-1, and C_4-C_5· bond of AB-2 had been cleaved, and that a new bond with C_5 of dihydronoracronycine (16) and, as necessary, a rearrangement had occurred. Fig. 7 indicates a plausible mechanism for this process in the case of 7 and 16. A similar scheme can be written for the reaction of AB-2 (9) and 16. Additionally, in the coupling reaction at room temperature, partial cleavege (about 50%) of the C_1-C_5· junction of AB-1 was estimated because dihydro AB-1 (17) and dihydro AB-2 (18) were each obtained in about 40% yield, respectively[20].

Because, when AB-1 (7) or AB-2 (9) was refluxed with methanolic HCl for 8h, these compounds were only partially destroyed to give noracronycine (6), or 6 and isonoracronycine (8), respectively. Dihydronoracronycine (16) must therefore perform a very important role in the cleavage reaction of AB-1 (7) and AB-2 (9) described above.

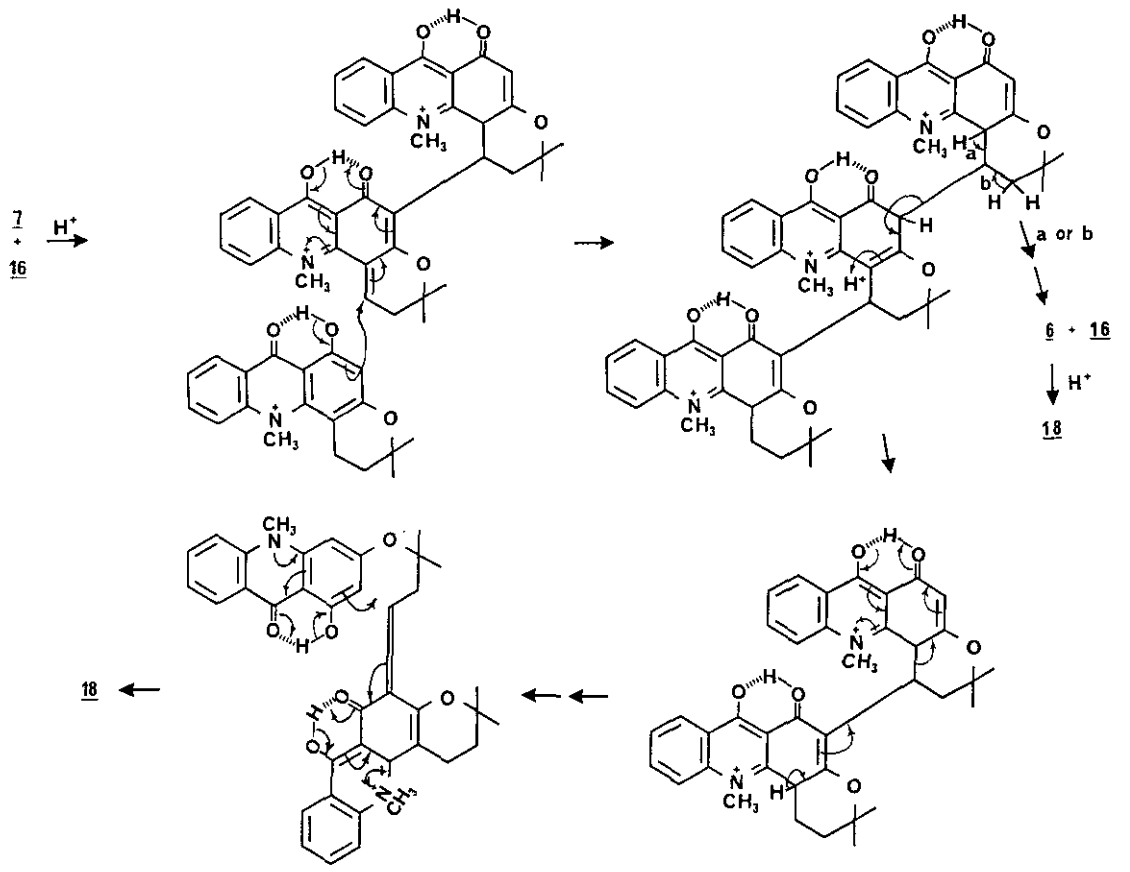


Fig. 7. Formation of dihydro AB-2 (18) from AB-1 (7).

Dihydro AB-1 (17) could be synthesized by the catalytic hydrogenation of AB-1 (7) or through the coupling of noracronycine (6) and dihydronoracronycine (16) at room temperature. Through the experiments described it was found, unexpectedly, that dihydro AB-1 (17) could also be synthesized by coupling AB-1 (7) and dihydronoracronycine (16) at room temperature.

Three procedures had already been described (4.1.1) for the formation of dihydro AB-2 (18), namely, catalytic hydrogenation of AB-2 (9), coupling of noracronycine (6) and dihydronoracronycine (16) at reflux, and coupling of 6 and 16 at room temperature. Subsequently, it was shown that dihydro AB-2 (18) could be produced by coupling AB-1 (7) or AB-2 (9) with dihydronoracronycine (16) under reflux with methanolic HCl.

It was estimated that in the coupling reaction described above (4.1.1), dihydro AB-2 (18) was synthesized in good yield (86%) owing to the reaction mechanism described herein, namely that AB-1 (7) and/or AB-2 (9), partially obtained through the reaction were also transformed into dihydro AB-2 (18) by the coupling of 7 or 9 with dihydronoracronycine (16).

4.2. Selective Synthesis of Various Oligomers through the Polymerization of Noracronycine (6)

4.2.1. Selective Synthesis of AB-1 (7)

For the purpose of improving the yields of the all angular compounds such as AB-1 (7), AB-3 (10), AB-4 (11) etc., 98% H_2SO_4 was used on noracronycine (6) instead of methanolic HCl at room temperature. Surprisingly, only AB-1 (7) was formed under these conditions, albeit in very low (4%) yield[23]. No reaction was observed and only unreacted acronycine (1) was recovered when 1 was treated with 98% H_2SO_4 at room temperature.

An improved yield of AB-1 (7) was obtained when noracronycine (6) was treated with methanolic H_2SO_4 (98% H_2SO_4-MeOH, 2:5, v/v) instead of 98% H_2SO_4 at room temperature under a N_2 atmosphere. This prompted us to study the reaction of noracronycine (6) in methanolic H_2SO_4 using ten different solvent ratios. Yields of AB-1 (7) were optimized when 98% H_2SO_4-MeOH in the ratio 1:1 or 2:3 was used. When noracronycine (6) was treated with a mixture of 98% H_2SO_4 and MeOH in the ratio 1:1, the yield of AB-1 (7) was about 39%, a tenfold increase compared with 98% H_2SO_4[23].

4.2.2. Preparation of AB-3 (10) and AB-4 (11)

During these studies, it was found that AB-3 (10), the angular-angular-angular trimer of noracronycine (6), was formed in fairly good yield (20%), together with AB-1 (yield 19%) and AB-4 (yield 9%), by successively treating 6 with a 9:1 and a 1:9 mixture of 98% H_2SO_4 and MeOH at room temperature[25,27].

4.2.3. Preparation of AB-1 (7) and AB-2 (2)

Interestingly, when noracronycine (6) was refluxed on a steam bath with p-toluenesulfonic acid in CH_2Cl_2 for 6h, AB-1 (7) and AB-2 (9) were obtained in fairly good yields (16% and 45%, respectively)[23].

5. STUDIES ON THE REACTION MECHANISMS USING DEUTERIUM-LABELED REAGENTS

5.1. Studies on the reaction mechanisms using deuterium-labeled reagents.

As described above, it was found that AB-1 (7), an angular-angular dimer, could be selectively synthesized (39% yield) by treating noracronycine (6) with a mixture (1:1) of MeOH and 98% H_2SO_4 at room temperature under a N_2 atomosphere (4.2.1). On the other hand, when a 1:10 mixture of noracronycine (6) and dihydronoracronycine (16) was refluxed on a steam bath with a mixture of MeOH-12 N aqueous HCl (5:2), dihydro AB-2 (18), a linear-angular dimer, was obtained in 86% yield (4.1.1). In order to clarify the mechanism involved in these two dimeri-

zation reactions, they were performed using a mixture of CH_3OD and 98% aqueous (D_2O) D_2SO_4 and a mixture of CH_3OH-12 N aqueous (D_2O) DCl, respectively[24]. It was estimated that under these reactions, protonation at the C_2 and C_6 positons would occur (Fig. 8), and the C_2-H and C_5-H would be exchanged to become C_2-D and C_5-D.

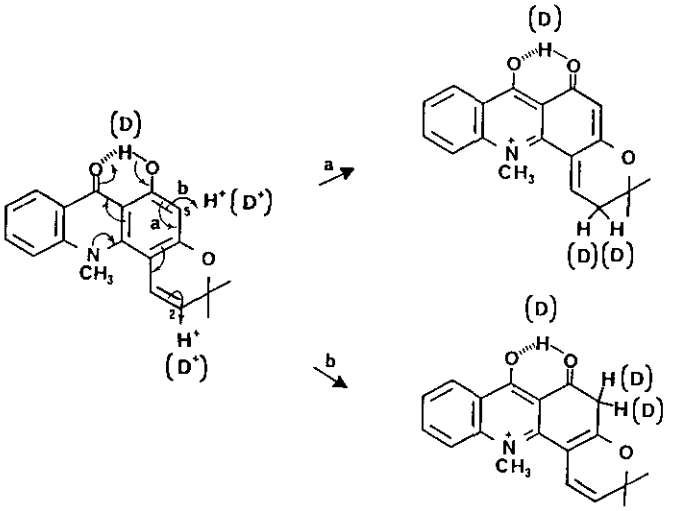


Fig. 8. Protonation of noracronycine (6) in acidic condition.

5.1.1. Formation of AB-1 (7): Dimerzation of Noracronycine (6) with 98% D_2SO_4-CD_3OD (1:1)

Noracronycine (6) was dissolved in a mixture (1:1) of CD_3OD and 98% aqueous (D_2O) D_2SO_4 and the solution was stirred under N_2 at room temperature. After two days, the reaction mixture was diluted with H_2O and extracted twice with $CHCl_3$. The combined $CHCl_3$ layers were successively washed with H_2O, 5% aqueous $NaHCO_3$ and H_2O. The two products were shown by comparison with authentic samples to have the same chromatographic mobility as noracronycine (6) and AB-1 (7)[24].

The unreacted noracronycine was evaluated by ms and ^{1}H-nmr. The M^+ at m/z 309 was 2 mass units higher than that of noracronycine (6, M^+ 307). In the ^{1}H-nmr spectrum, no C_2-methine signal was observed, and the C_5-H singlet was reduced to about a quarter of its original intensity. In agreement with these changes, the C_1-H doublet (δ 6.565, J = 9.6 Hz)[16] was simplified to a singlet. From the accumulated data, noracronycine-d_2 was demonstrated to have the structure 24[24].

On the other hand, the mass spectrum of AB-1 indicated an M^+ at m/z 618, 4 mass units higher than that of AB-1 (7, m/z 614) obtained using a mixture (1:1) of CH_3OH and 98% aqueous (H_2O) H_2SO_4[23]. Location of the deuterium label was determined through nmr spectroscopy. In the ^{1}H-nmr spectrum, the C_2-H_2 and the $C_{2'}$-H signal were not observed, and the C_5-H was substantially (ca. 80%) reduced in intensity. As expected from these changes, the dibenzylic proton (C_1-H), originally observed as a doublet of doublets (J = 7.3 and 11.7 Hz), and the $C_{1'}$-H originally observed

as a doublet (J = 9.6 Hz)[17,23], were reduced in complexity to singlets at δ 5.153 and 6.170, respectively. Hence, the structure of AB-1-d_4 was established to be 25, in which the increase of four mass units is readily explained.

Because the deuterium incorporation at C_2-H of noracronycine (6) was essentially 100%, it was estimated that equilibrium reaction a (Fig. 8) developed rapidly. At the same time, exchange at C_5 was also occurring (reaction b). If the second noracronycine unit is trapped instead of H^+ (D^+), dimerization occurrs to afford AB-1. From these observations, the reaction mechanism for the formation 24 and 25 was elucidated as shown in Fig. 9.

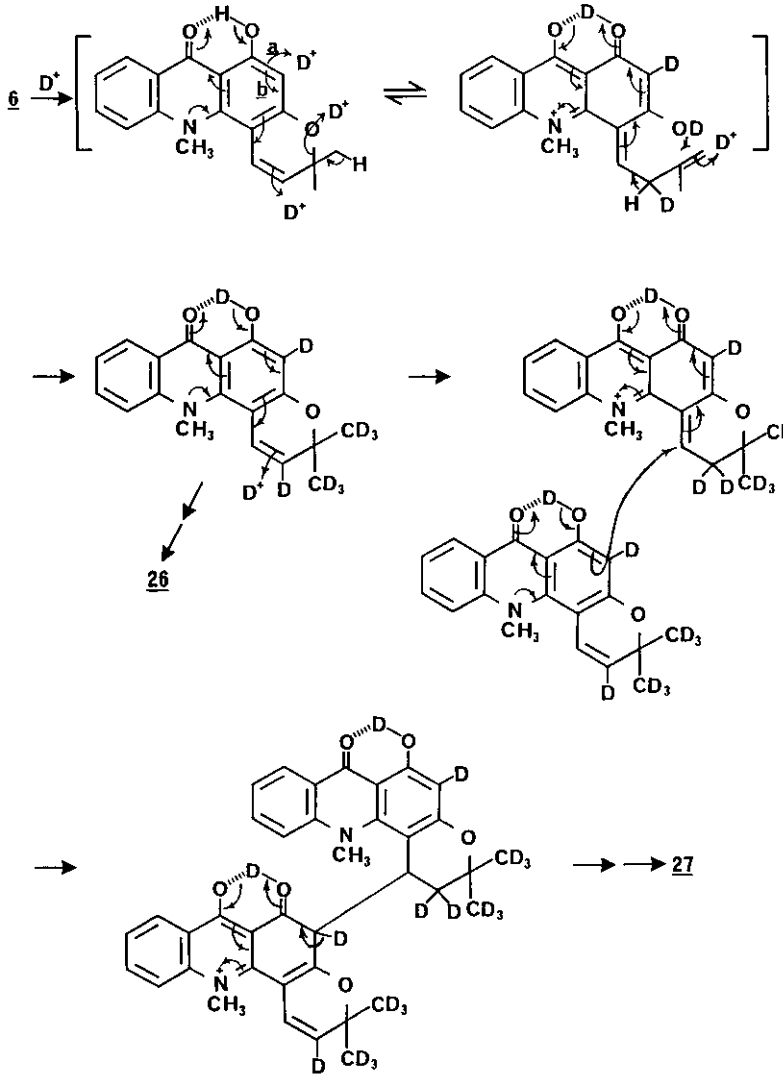
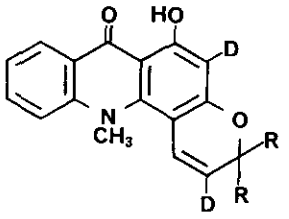


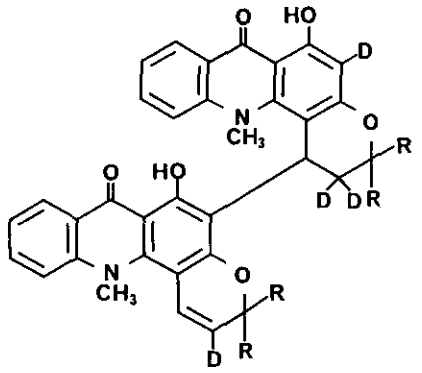
Fig. 9. Mechanism of formation of noracronycine-d_8 (26) and AB-1-d_{16} (27).

Surprisingly, noracronycine-d_8 (26) and AB-1-d_{18} (27) were obtained, instead of 24 and 25, when D_2O was used throughout the dilution-extraction work-up procedure. Therefore, it was estimated that a ring-opening rection of the chromane ring must occur and that all of the geminal methyl hydrogens had been exchanged by deuterium (Fig. 9)[24].



24 R = CH_3

26 R = CD_3



25 R = CH_3

27 R = CD_3

^{1}H-Nmr spectra of noracronycine (6) and noracronycine-d_8 (26) are compared in Fig. 10[28]. In the ^{1}H-nmr spectrum of the latter compound, signals attributed

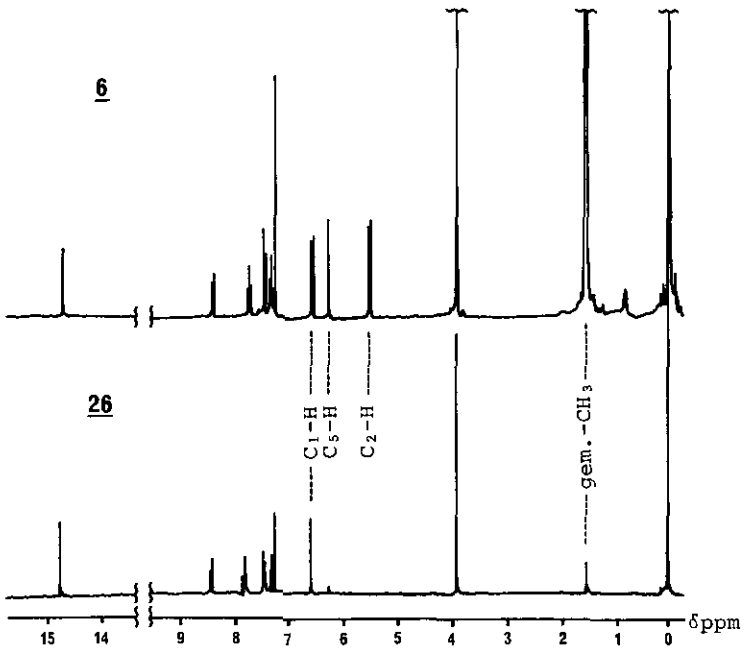
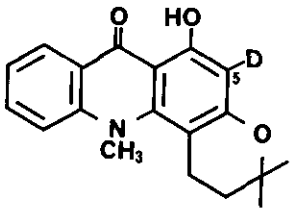


Fig. 10. Comparison of ^{1}H nmr spectra of noracronycine (6) and noracronycine-d_8 (26) in $CDCl_3$.

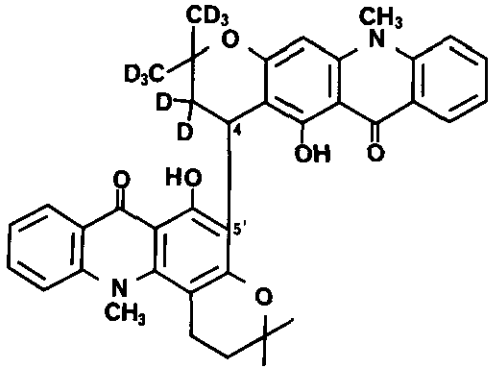
to geminal methyls, C_2-H and C_5-H have disappeared or decreased significalntly. Because exchange was observed not only at the C_2 and $C_{2'}$ positions, but also at C_5-H and the geminal methyl groups, it was thought that when the reaction was complete, all of these postions were exchanged by D, but through dilution with H_2O (rather than D_2O), the geminal CD_3 and part of the C_5-D were re-exchanged to CH_3 and and C_5-H, respectively.

5.1.2. Formation of Dihydro AB-2 (18): Dimerization of Noracronycine (6) and Dihydronoracronycine (16) with 12N Aqueous (D_2O) DCl and CH_3OD (1:2.5)

When a 1:10 mixture of noracronycine (6) and dihydronoracronycine (16) was refluxed with methanolic HCl, a linear-angular type dimer, dihydro AB-2 (18), was obtained in 86% yield[17,23]. To clarify this reaction mechanism, the same reaction was conducted using a 1:2.5 mixture of 12N aqueous (D_2O) DCl and CH_3OD[24]. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. Two compounds, chromatographically identical with dihydronoracronycine (16) and dihydro AB-2 (18), were isolated by preparative tlc. As expected, the M^+ of the dihydronoracronycine obtained appeared at m/z 310, one mass unit higher than 16. In its ^{1}H-nmr spectrum, the aromatic singlet at δ 6.17 was reduced in intensity by about 80%. No other changes were observed. Consequently, the structure of dihydronoracronycine-d_1 was 28 where deuterium was incorporated at C_5.



28



29

On the other hand, dihydro AB-2 (18) was regarded as being formed by the rearrangement of dihydro AB-1 (17). As a result, during the formation of deuterodihydro AB-1, it was anticipated that three deuteriums would be introduced, at the C_2-methylene and at C_5-H. In the process of the rearrangement, deuterodihydro AB-1 would lose the C_5-D and gain a deuterium at C_{12} to afford a dihydro AB-2-d_3. Such a compound would have an estimated M^+ at m/z 619. However, the observed M^+ of deuterodihydro AB-2 was at m/z 624, an increase of 8 mass units. Examination

of the ^{1}H nmr spectrum of this compound showed that the C_3-methylene was fully deuterated, and surprisingly, a set of geminal methyl signals had also disappeared. In the ^{1}H-nmr spectrum of dihydro AB-2 (18), four methyl signals assigned to the two geminal signals were observed, namely, δ 0.700, 1.157, 1.435 and 1.495 (each 3H, s)[17,23]. But in the ^{1}H-nmr spectrum of the d_8 derivative of AB-2 obtained herein, only two such signals were observed at δ 0.703 and 1.161, which had previously been assigned[17] to the geminal methyl signals of the lower, angular unit of dihydro AB-2 (18). No deuterium incorporation at the C_5 position was observed, and the structure of deuterodihydro AB-2 was therefore defined as 29[24]. Consequently, in addition to the rearrangement mechanism, a ring-opening mechanism may also be occurring during the formation of dihydro AB-2-d_8 (29).

The observation that the C_5 position of deutero AB-2 was not labeled, even though it should be replaced by D during the rearrangement reaction, suggested that the D exchanged at this position might be replaced by H during the work-up process. Through these experiments, it was also found that the geminal methyls of the upper unit of deuterodihydro AB-2 (29) after work-up using H_2O are still CD_3. Therefore, it appears that even after the dilution with H_2O, no reexchange of these upper geminal CD_3 to CH_3 takes place in the case of 29 (Fig. 11).

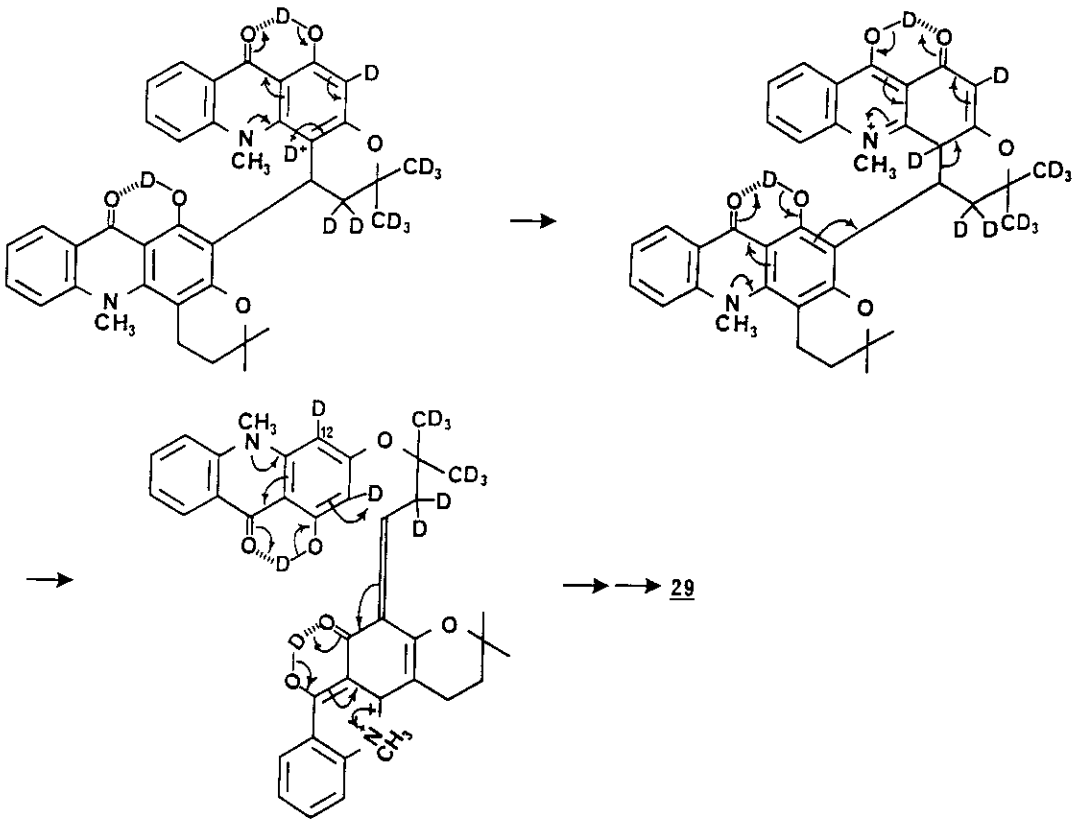


Fig. 11. Rearrangement of dihydro AB-1-d_{11} to dihydro AB-2-d_8 (29).

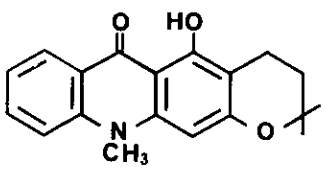
6. TRANSFORMATION OF ACRONYCINE (1) TO ISOACRONYCINE (3)

6.1. Reaction of Dihydronoracronycine (16) with 98% H_2SO_4

In the course of our continuing study of the chemistry of acronycine, we had the opportunity to examine the products of the reaction between acronycine (1) and dihydronoracronycine (16) with 98% H_2SO_4 at room temperature. Whereupon it became apparent that acronycine was not involved in the reaction and that the same reaction products were obtained by treating dihydronoracronycine (16) alone with 98% H_2SO_4 at room temperature[22].

One of the reaction products displayed ir, uv and ms properties comparable to those of dihydronoracronycine (16). In the ^{1}H-nmr spectrum, a geminal methyl singlet (δ 1.404), a N-CH_3 signal (δ 3.786), an aromatic singlet (δ 6.293) and a hydrogen-bonded phenolic proton (δ 15.127) were observed in addition to four coupled aromatic protons and two pairs of methylene protons[22].

The structure of this product was deduced on the basis of transient nuclear Overhauser experiments. Thus, when the three-proton singlet for the N-methyl group at δ 3.766 was irradiated, an enhancement was observed not only in the doublet of doublets at δ 7.464 (19%), but also in the singlet at δ 6.293 (22%). Significantly, no enhancement was observed in the methylene group at δ 2.775 (2H, t, J = 6.8 Hz). This compound, therefore, cannot have the angular structure of 16, but must have the linear structure found in isonoracronycine (8)[18]. The structure of the product, was, therefore, envisaged as dihydroisonoracronycine (30). Direct comparison with dihydroisonoracronycine (30) formed by the catalytic

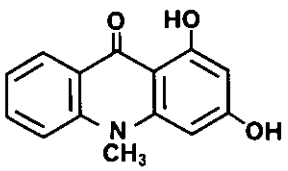


30

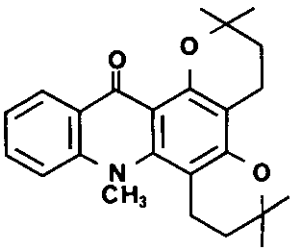
hydrogenation of isonoracronycine (8), established the identity. Confirmation of the structure was achieved when 30 was found to undergo dehydrogenation to 8 with DDQ at room temperature for two days[22].

It is worth noting that through these procedures it is possible to achieve the transformation of acronycine (1) to isoacronycine (3) because 3 was obtained by treating 8 with $(CH_3)_2SO_4$/acetone, K_2CO_3 at refluxing conditions.

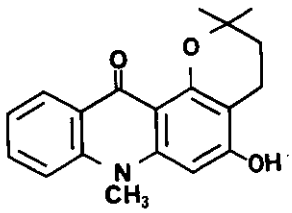
As well as 30, 1,3-dihydroxy-10-methylacrid-9-one (31), 32 and 33 were also obtained from the reaction mixture described above. Through these observations the possibility of an intermolecular rearrangement in the transformation of dihydronoracronycine (16) to dihydroisonoracronycine (30), was considered which leads



31



32



33

to disproportionation and the formation of dihydroisonoracronycine (30), 1,3-dihydroxy-10-methylacrid-9-one (31), 32 and 33 (Fig. 12)[26].

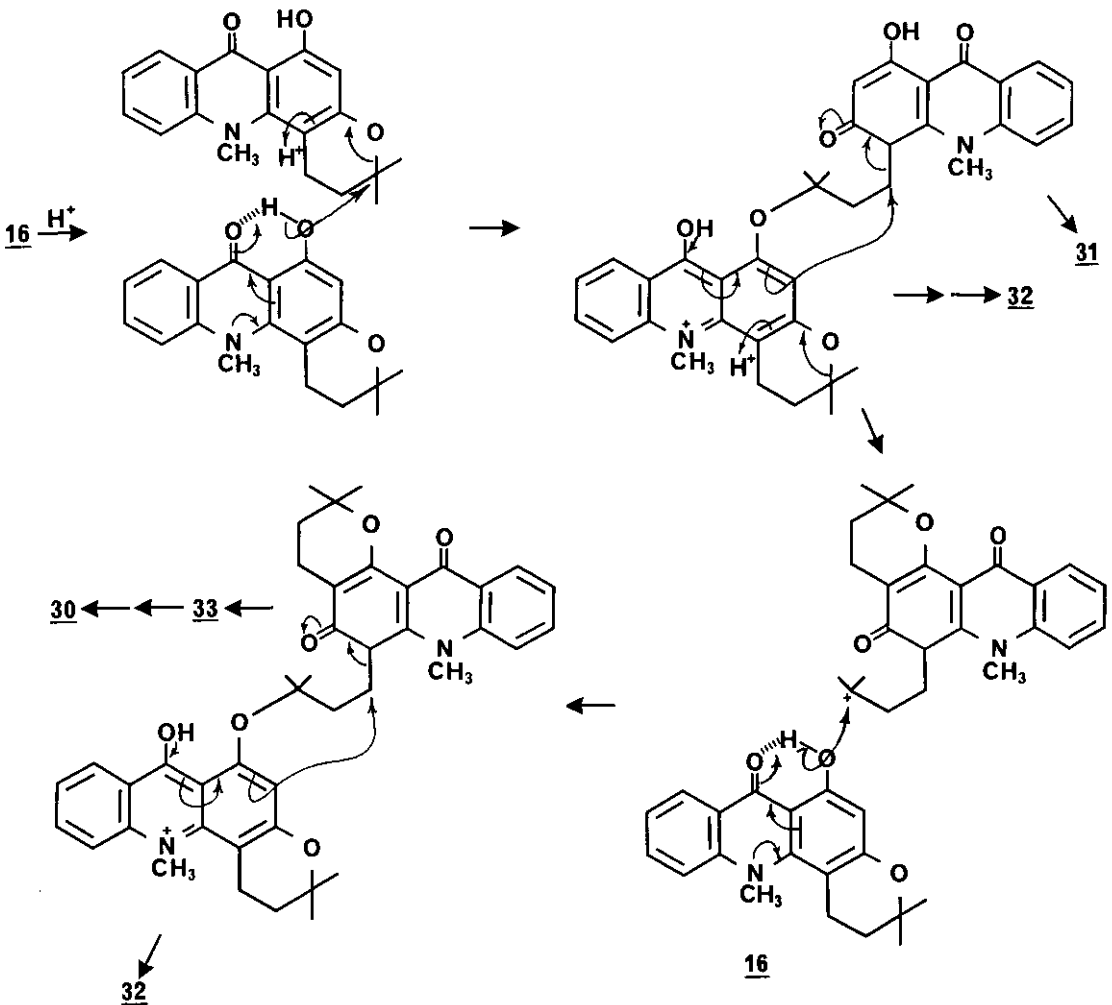
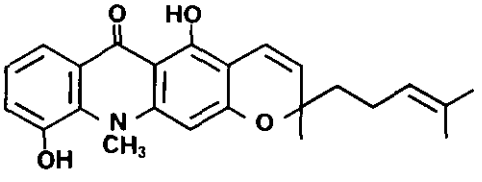


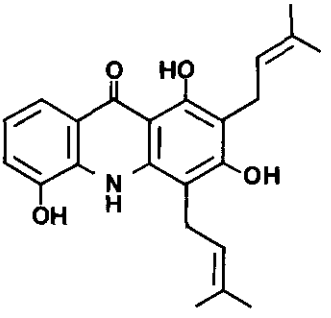
Fig. 12. Formation of compounds 30 - 33.

It is interesting that acronycine (1), noracronycine (6) and dihydronoracronycine (16) exhibit quite distinct reactions with 98% H_2SO_4. When 1 was treated with 98% H_2SO_4, no reaction was observed[22]. On the other hand, a dimer was obtained by treating noracronycine (6) with 98% H_2SO_4[23], and dihydroisonoracronycine (30), 1,3-dihydroxy-10-methylacrid-9-one (31), 32, and 33 were obtained by treating dihydronoracronycine (16) with 98% H_2SO_4[26].

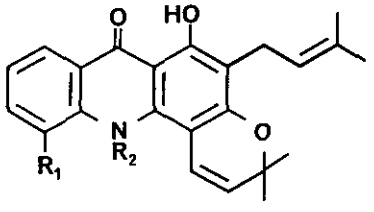
Because the compounds 30, 32 and 33 possess the same skeleton as the known naturally occurring acridones glycofoline (34)[29], atalaphylline (35)[30], 36[31], severifoline (37)[32] and 38[33], the reactions described herein are interesting from the view point of the total syntheses of these naturally occurring acridone alkaloids.



34

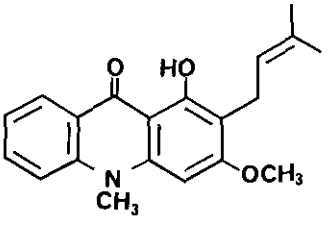


35



36 $R_1 = OH$, $R_2 = CH_3$

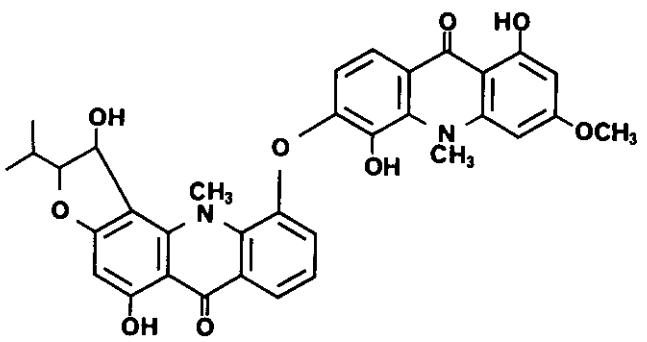
37 $R_1 = R_2 = H$



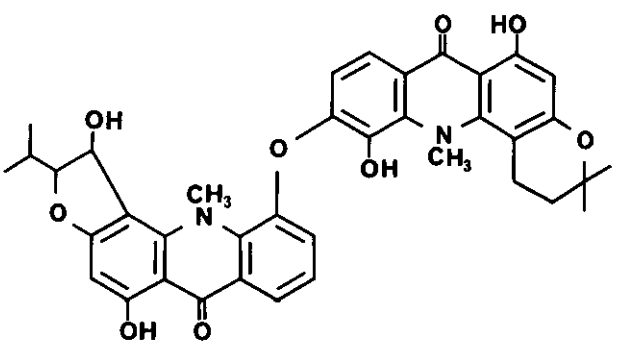
38

7. NATURALLY OCCURRING ACRIDONE DIMERS

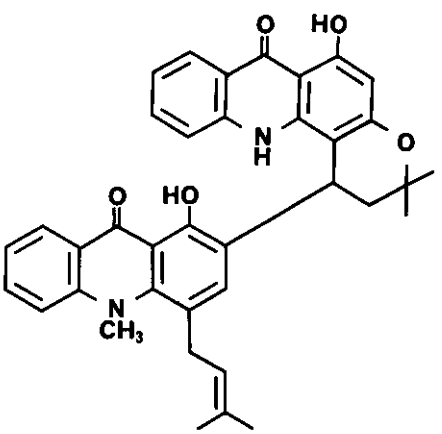
Dimers of acridone alkaloids, atalanine (39) and ataline (40), which are the dimers *via* an ether linkage, have been isolated from *Atalantia ceylanica* (Rutaceae)[34]. Whereas the first acridone dimers *via* a C-C linkage are the compounds described herein. After reporting on these acridone dimers, glycobismine A (41) was reported as a C-C dimer obtained from the root bark of *Glycosmis citrifolia* (Rutaceae)[35].



39



40



41

8. CYTOCIDAL ACTIVITIES OF ACRONYCINE (1) AND RELATED COMPOUNDS

Because it was demonstrated that acronycine (1) possessed the widest spectrum of antitumor activities agaist various kinds of murine tumors[4-6], we were consequently interested in the antitumor activities of the acridone derivatives obtained through these experiments. Cytocidal activities against P-388 cells (ED_{50}) of some of the acridone derivatives described herein are as follows: 1, 2.4 μg/ml; 3, 4.2 μg/ml; 6, 50.0 μg/ml; 7, 36.4 μg/ml; 8, >50.0 μg/ml; 9, 72.0 μg/ml; 10, >50.0 μg/ml; 16, 2.8 μg/ml; 17, 61.0 μg/ml; 18, 1.3 μg/ml; 19, 5.3 μg/ml; 20, 4.2 μg/ml; 21, 47.0 μg/ml; 22, >50.0 μg/ml and 30, 10.8 μg/ml. Interestingly some of these compounds exhibited stronger cytocidal activities than that of 1 and *in vivo* evaluation of some of these compounds will be conducted subsequently.

9. CONCLUSION

By treating acronycine or noracronycine with methanolic hydrochloric acid, various kinds of oligomers were obtained, including several compounds possessing a rearranged partial structure. We have also succeeded in the selective syntheses of monomers and/or oligomers of noracronycine and related compounds by altering the reaction condition(s). Further, polymerization and/or rearrangement reaction mechanisms were defined by the combination of the reactions using D-substituted reagents and ^1H nmr spectroscopic analysis. Because very little is known about the chemical characteristics of acridone alkaloids these experimental results should prove useful in further studies on this group of compounds.

ACKNOWLEDGEMENTS

The authors would like to thank Drs. G.H. Svoboda and M. Gorman (formerly of Lilly Research Laboratories) for samples of acronycine, Drs. H. Wagner and H.L. Lotter (Universität München) for the X-ray analyses of AB-1 and AB-2, the Midwest Regional NMR Facility (University of Illinois at Urbana) for nmr spectra, Dr. J.R. Lewis (University of Aberdeen) and Dr. I. Mester (Universität Münster) for authentic samples, Drs. R.D. Macfarlane and C.J. McNeal (Texas A & M University) for ^{252}Cf plasma desorption mass spectrometry, Drs. R.P. Borris, C.-T. Che, C.M. Compadre, L.-J. Lin and G.T. Marshall (The University of Illinois at Chicago) for nmr and ms spectra, Dr. J.M. Pezzuto (The University of Illinois at Chicago) for *in vitro* bioassays and the Division of Cancer Treatment, National Cancer Institute (USA) for a grant (CA-20164).

REFERENCES

1. G.K. Hughes, F.N. Lahey, J.R. Price, and L.J. Webb, Nature, 1948, 162, 223.
2. F.N. Lahey and W.C. Thomas, Aust. J. Sci. Res., 1949, 2A, 423.
3. G.A. Cordell, in "The Alkaloids" vol. 25., Ed. A. Brossi, Academic Press, New York, N.Y., 1985, p. 38.
4. G.H. Svoboda, J. Pharm. Sci., 1966, 55, 758.
5. K. Gerzon and G.H. Svoboda, in "The Alkaloids" vol. 21., Ed. A. Brossi, Academic Press, New York, N.Y., 1983, p. 1.
6. J. Reish and S.M. El-Moghazy Aly, Archiv. Pharm., 1986, 319, 25.
7. R.D. Brown, L.J. Drummond, F.N. Lahey, and W.C. Thomas, Aust. J. Sci.Res., 1949, 2A, 622.
8. L.J. Drummond and F.N. Lahey, Aust. J. Sci. Res., 1949, 2A, 630.
9. R.D. Brown and F.N. Lahey, Aust. J. Sci. Res., 1950, 3A, 593.
10. P.L. McDonald and A.V. Robertson, Aust. J. Chem., 1966, 19, 275.
11. T.R. Govindachari, B.R. Pai, and P.S. Subramaniam, Tetrahedron, 1966, 22, 3245.
12. T.R. Govindachari, B.R. Pai, and V.N. Ramachandran, Ind. J. Chem., 1968, 6, 179.
13. J.Z. Gougoutas and B.A. Kaski, Acta Crystallogr., 1970, 26B, 853.
14. J.R. Beck, R.N. Booher, A.C. Brown, R. Kwok, and A. Pohland, J. Am. Chem. Soc., 1967, 89, 3934.
15. W.D. Crow and J.R. Price, Aust. J. Sci. Res., 1949, 2A, 255.
16. S. Funayama, R.P. Borris, and G.A. Cordell, J. Nat. Prod., 1983, 46, 391.
17. S. Funayama, G.A. Cordell, H. Wagner, and H.L. Lotter, J. Nat. Prod., 1984, 47, 143.
18. S. Funayama and G.A. Cordell, Planta medica, 1983, 48, 263.
19. S. Funayama and G.A. Cordell, J. Nat. Prod., 1984, 47, 285.
20. S. Funayama and G.A. Cordell, Heterocycles, 1983, 20, 2379.
21. S. Funayama and G.A. Cordell, Planta medica, 1984, 50, 121.
22. S. Funayama and G.A. Cordell, J. Nat. Prod., 1985, 48, 114.
23. S. Funayama and G.A. Cordell, J. Nat. Prod., 1985, 48, 536.
24. S. Funayama and G.A. Cordell, J. Nat. Prod., 1985, 48, 547.
25. S. Funayama, G.A. Cordell, R.D. Macfarlane, and C.J. McNeal, J. Org. Chem., 1985, 50, 1737.
26. S. Funayama and G.A. Cordell, J. Nat. Prod., 1985, 48, 938.
27. S. Funayama and G.A. Cordell, J. Nat. Prod., 1986, 49, 210.
28. S. Funayama and G.A. Cordell, Unpublished results.
29. T-S. Wu and H. Furukawa, Heterocycles, 1982, 19, 825.
30. D. Basu and S.C. Basa, J. Org. Chem., 1972, 37, 3035.
31. A.W. Fraser and J.R. Lewis, J. Chem. Soc, Perkin I, 1973, 1173.
32. T.-S. Wu, C.-S. Kuoh, and H. Furukawa, Phytochemistry, 1982, 21, 1771.
33. K. Rastogi, R.S. Kapil, and S.P. Popli, Phytochemistry, 1980, 19, 945.
34. A.W. Fraser and J.R. Lewis, Chem. Commun., 1973, 615.
35. H. Furukawa, T.-S. Wu, C.-S. Kuoh, T. Sato, Y. Nagai, and K. Kagei, Chem. Pharm. Bull., 1984, 32, 1647.

Received, 13th October, 1988