

THE N-O-O TRIANGULATION HYPOTHESIS -- AN ASSESSMENT AFTER ONE DECADE

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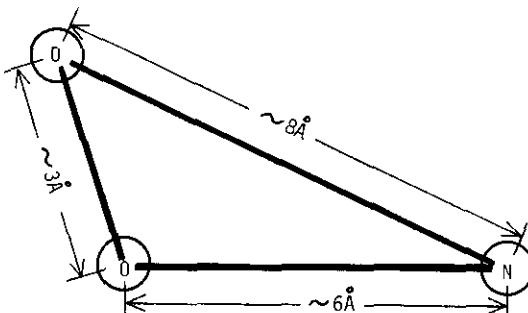
A triangular atomic arrangement composed of three electronegative atoms (one nitrogen and two oxygen) was observed among many antineoplastic compounds in 1970. Today, the extent of the application of this hypothesis is assessed with affirmative and nonconfirmative examples provided from the literature. The hypothesis has been used for the design and synthesis of several interesting antineoplastic agents, including DHAQ.

A story is told that during a war period, a group of Indian soldiers were lost in the Burma jungle. After several week's search in vain by their comrades, the hope of their return gradually vanished. Yet a couple months later, these Indian soldiers returned to their headquarters tired, but otherwise unharmed. They were asked by their commander-in-chief how they found their way back. A battered map was proudly presented to him. On close examination, the map was one of a suburb of Zurich, Switzerland! Somehow from that map and with faith, these soldiers reached their goal.

The reason for telling this story is that in the jungle of cancer research, we still have a great deal to learn; but we also have a need for maps to be drawn, be they correct or incorrect as long as they were drawn earnestly and with proper rationale. Thus the explorers can take their first and second steps and, with the maps continually redrawn, perhaps one of these days the goal can be reached.

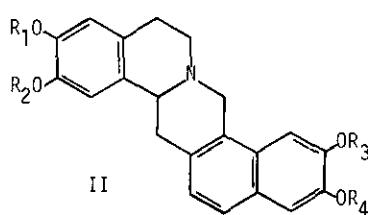
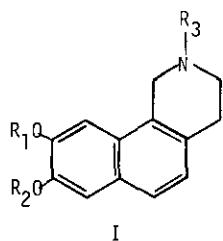
Ten years ago, in connection with our structure-activity study of various antineoplastic agents, a triangular atomic arrangement composed of three electronegative atoms (one nitrogen and two oxygen), separated from one another at appropriate interatomic distances, was noted among a number of compounds possessing experimental antileukemic activity. The N-O-O triangular pattern was put forward as a working hypothesis for the continued search for antineoplastic agents (1). In the original communication, interatomic distance measurements of alkaloids such as tylocrebrine, tylophorine, camptothecin, demecolcine, vinblastine, vincristine, harringtonine and emetine; of antibiotics such as streptonigrin, anthramycin, daunomycin and actidione; and of

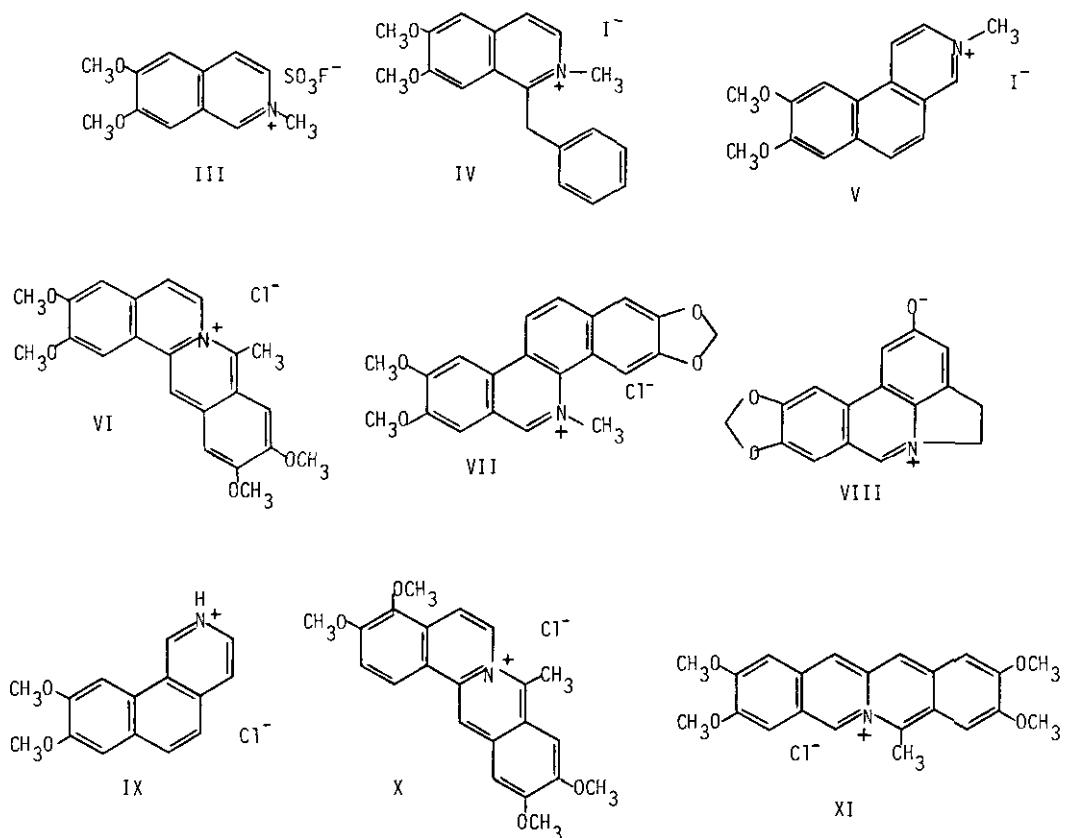
synthetic compounds such as the folic acid antagonists and several purine and pyrimidine nucleosides, were measured from their molecular models constructed on the basis of available information of their spatial conformation.



As stated in the original communication, the observation was rather empirical, the facts presented were oversimplified and the triangular pattern could not be applied to include the structures of other antileukemic compounds such as hydroxyurea, ellipticine, or biological alkylating agents. Nevertheless, the feature was postulated as a contributing factor in the binding to one of the pertinent receptor sites in certain biopolymers involved in leukemia genesis and was presented to be used in the exploration of *in vivo* drug interaction in greater detail as well as in designing better and more useful antineoplastic agents. Thereafter, Adamson (2) suggested that compounds with this triangular pattern may simply share a common transport system into neoplastic cells where each compound can then exert its biological action against the target cells.

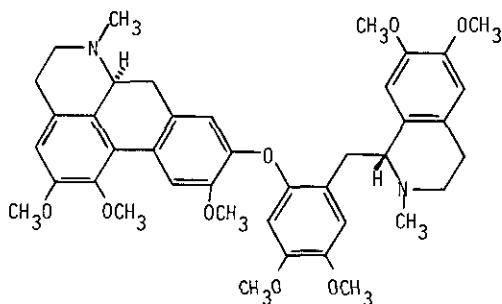
Based on the preceding concept, synthesis of several dialkoxytetrahydrobenzo[*b*]isoquinolines (I) and tetraalkoxytetrahydrobenzo[*a*]naphtho[1,2-*g*]quinolizines (II) was initially conducted (3). Although a few derivatives, such as 2,3-dimethoxy-7,8,13b,14-tetrahydro-5*H*-[1,3]benzodioxolo[5,6-*a*]naphtho[1,2-*g*]quinolizine hydrochloride (II, $R_1+R_2=OCH_2O$; $R_3, R_4=CH_3$, HCl salt), did show inhibitory activity against leukemia P388 in mice, the activity was not high enough to warrant further study. Subsequently, it was found that fully aromatized isoquinolines substituted with proper alkoxyl groups, such as compounds III - V, possessed higher activity against leukemia P388. This eventually led to the discovery of antileukemic activity of coralyne (4,5) (VI), nitidine (6) (VII), ungeremine (7) (VIII), and related condensed isoquinolinium salts (8) (IX - XI).





The interatomic distance between one of the oxygen atoms and the nitrogen atom in the alkoxyisoquinoline series is somewhat shorter ($\sim 7\text{\AA}$ instead of $\sim 8\text{\AA}$) than the previously reported value. It would thus suggest that the originally designated distances between these electronegative atoms be modified or the interatomic limiting distance range be slightly broadened. Since it is known that intermolecular bonding involving electronegative atoms, such as hydrogen bonding, allows some latitude in configuration (9), within reasonable limits, exact matching of distances in receptor and drug may not be necessary, and drugs where these distances vary may still be active (10).

The N-O-O triangulation hypothesis has also been evaluated by other investigators. The first confirmation of the conjecture was the report by Allen and Creaven (11) wherein the investigators stated that the part of the novel antitumor alkaloid thalicarpine molecule (XII) necessary for antineoplastic activity is the aporphine moiety with part of the benzylisoquinoline group. This corresponds with the proposed N-O-O triangulation feature. Cushman et al (12)

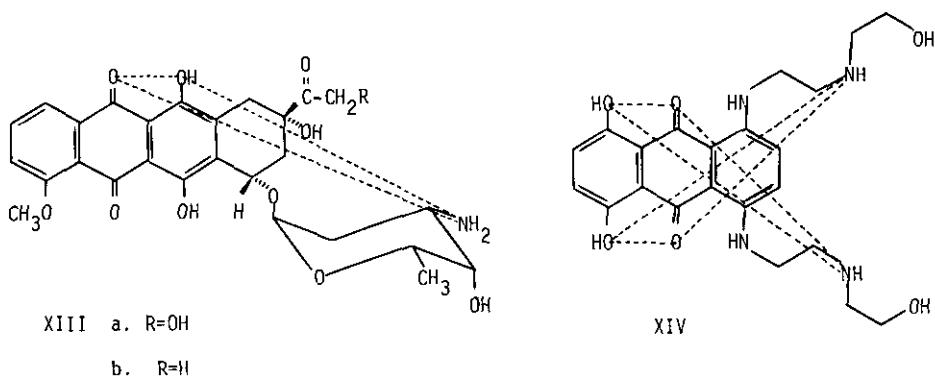


XII

conducted a conformation study of protoberberine alkaloids and concluded that the structural feature requirements for compounds in connection with the inhibitory activity against leukemia P388 were in accord with our observation. Mawdsley et al (13) reported that the correlation coefficient calculation between a number of polymethoxy-substituted heterocyclic compounds and their biological activity agreed with our proposed triangulation feature. Stermitz et al (14) prepared a series of chelerythrine and sanguinarine derivatives with one N-O bond distance much shorter than that in the nitidine series (6) and failed to observe antileukemic activity. In contrast, fagaronine, a close analog of nitidine wherein the methylenedioxy group of the latter compound is replaced by a hydroxy and a methoxy group, possesses high activity against P388 leukemia in mice (15). There are also reports from several investigators who, based on studies with their compounds, did not support our original proposition: Townsend et al (16), in the study of a number of nucleosides, ruled out the possible application of the proposed feature to compounds in that area. Kingston and Sami (17), using the triangulation concept, designed several modified indole alkaloids of the iboga series and reported that the cytotoxicity of these compounds was not significantly increased over the unmodified alkaloids. An X-ray diffraction study of anthramycin crystals by Mostad et al (18) indicated that the fit to the proposed triangulation pattern (which they believed may be of importance to the biological transport mechanism) is rather poor. It should be pointed out, however, that the conformation of a molecule may differ in the crystal phase, in solution, and when bound to a biological receptor (19). On the other hand, if all the pertinent electronegative atoms required to make up the triangulation unit were scattered over a non-rigid structure, the resulting molecule may be too flexible to form any meaningful and definitive structural pattern. This is illustrated by the report that Schulze et al (20), using the triangle concept, prepared a number of quaternary N-heterocyclic azomethines containing a bis(2-hydroxyethyl)amine moiety on the side chain. None of these azomethines possess any antineoplastic activity.

The anthracycline antibiotics adriamycin (XIIa, doxorubicin) and daunomycin (XIIb,

daunorubicin) are among the most important antineoplastic agents studied in recent years. However, these drugs are rather toxic. They not only produce stomatitis, alopecia, and bone marrow depression, but often can cause severe, cumulative and irreversible cardiac toxicity. Adamson (21), based on the fact that both drugs fit the N-O-O triangulation feature, proposed the removal of the aminosugar daunosamine portion from these molecules and replacing the amino function (which contains the pertinent nitrogen atom for completing the N-O-O triangulation feature) with an appropriate amino group at a proper spatial distance from the oxygen atoms on the aglycone moiety. Since chemical synthesis of these antibiotics is often complicated by the stereochemistry of the unaromatized ring of the tetracyclic aglycone, it was decided to eliminate that ring and have the amino function attached to the planar anthraquinone ring system. This resulted in the synthesis of 1,4-dihydroxy-5,8-bis{[2-(2-hydroxyethyl)amino]ethyl}amino-9,10-anthracenedione (22, 23) (XIV, DHAQ), which, of course, is no longer a heterocyclic compound. This compound possesses two of the proposed N-O-O triangulation features.

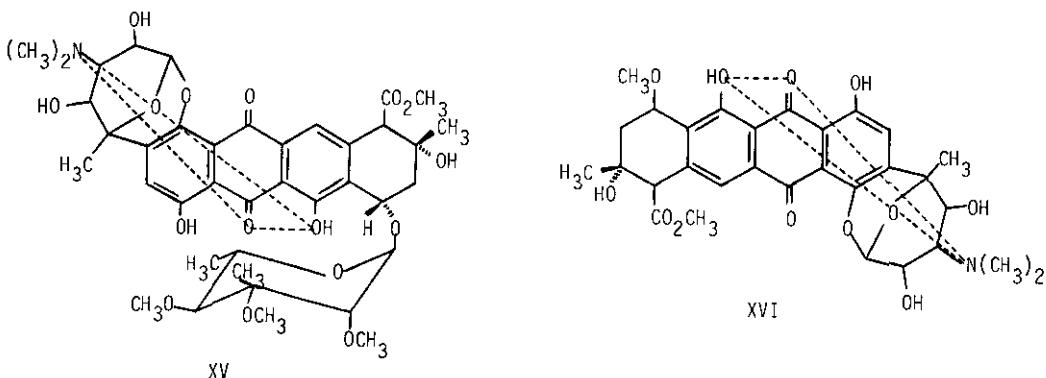


DHAQ exhibited excellent antineoplastic activity against leukemia P388 and L1210, against B16 melanocarcinoma, and against colon 26 in mice at low doses (22,23). It proved to be more effective than many standard anticancer agents including adriamycin, 5-fluorouracil, cyclophosphamide, methotrexate, vincristine, 1- β -D-arabinofuranosylcytosine or thio-TEPA against these experimental tumor systems (24) and is currently undergoing clinical trials (cardiotoxicity has not yet been observed). The importance of the nitrogen atom in the middle of the side chain of DHAQ can be readily demonstrated by the fact that replacement of this -NH- linkage by -S- or -CH₂- resulted in total elimination of the original antineoplastic activity (22).

The N-O-O triangulation feature is logically applicable to other antitumor anthracycline antibiotics possessing these electronegative atoms at the "strategic" positions. These include the baumycins (25), the aclacinomycins (26), pyrromycin (25), the cinerubins (27), carminomycin (28), kidamycin (29), the β -rhodomycins (30), the bohemic acid complex (musettamycin, marcello-mycin, rudolphomycin, alcindoromycin, collinemycin, and mimimycin) (31), and the roseorubicins

(32), among others. It is of interest to note that no antineoplastic activity has ever been reported for steffimycin B (33), an anthracycline antibiotic which binds to DNA but does not contain a nitrogen function in its sugar moiety.

Of particular interest is the antitumor antibiotic nogalamycin (34,35) (XV). Although its structure resembles those of the aforementioned anthracycline antibiotics, it differs from the rest in one important structure-activity aspect: The aglycone portion of adriamycin and related antibiotics does not retain the original antineoplastic activity without the sugar moiety attached; yet the neutral sugar substituted at the C₇ position of nogalamycin is not required for antineoplastic activity. This can best be illustrated by the fact that 7-*con*-O-methyl nogalitol (XVI) showed even better inhibitory activity against leukemia P388 than either nogalamycin or adriamycin (36). A study using the Dreiding molecular models showed that the N-O-O triangulation does exist in both the nogalamycin molecule and the O-methyl analog with the nitrogen atom located in the fused aminosugar portion, as illustrated in structures XV and XVI.



In recent years, one of the much discussed postulations on the mode of drug action is that certain drugs may bind to the double helical DNA by intercalation. Since many antineoplastic agents described by us contain, in a part of their molecules, a planar structure, the DNA intercalation concept should also be considered. In this regard, the aforementioned agents may arbitrarily be divided into two types. The first type includes those compounds which possess the alkoxyisoquinoline unit (e.g. structures III-XI). Compounds of this type are usually quite rigid and the triangulation is fixed in a planar or pseudo-planar ring system. The second type, which includes the anthracycline antibiotics and DHAQ, contains two immobile oxygen units with the nitrogen unit not fixed at one position. If DNA intercalation does take place, the nitrogen atom of compounds of the second type probably lies close to the deoxyribose-phosphate chain, permitting a strong interaction between the compound and DNA away from the intercalation site. It is to be noted that, although interaction and intercalation studies between DNA and agents

such as coralyne were reported (37,38), most of these studies were conducted in vitro, which may not necessarily reflect the actual mechanism of action that takes place in vivo. With our present limited knowledge in the binding of drugs to biological macromolecules, the possibility that many agents may initially bind to other biopolymers, such as plasma protein or cell surface, cannot be ruled out. It needs to be reemphasized that, in addition to the triangulation concept, other factors, such as geometric effects, electronic effects, lipophilicity, redox potentials, pKa, and in vivo stability and reaction rates of a compound should also be considered (1) for a more thorough understanding of drug action.

Horton, et al (39) prepared adriamycin and daunorubicin analogs wherein the amino function in the aminosugar daunosamine is replaced by an hydroxyl function. These compounds were reported active against leukemia P388, albeit at much higher doses than the parent antibiotics. This interesting finding suggests that a systematic investigation of the relevance of an oxygen atom in place of the nitrogen atom with regard to the N-O-O triangulation would be in order. This could be an intriguing idea for further study. The results can perhaps be incorporated with the structure activity relationship studies of alkoxyisoquinolines and DHAQ to furnish agents with better therapeutic value.

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