to these positively charged ions. These results support the finding expressed in Table II.

The investigation provided further information on the viscous nature of the salicylic acid–cetrimide system and its stability toward surfactants as additives.

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COMMUNICATIONS

Inhibitors of t-RNA *O*-Methyltransferase as Possible Antineoplastic Agents

Keyphrases □ t-RNA *O*-methyltransferase inhibitors—as potential antineoplastic agents □ Antineoplastic agents, potential—inhibitors of t-RNA *O*-methyltransferase

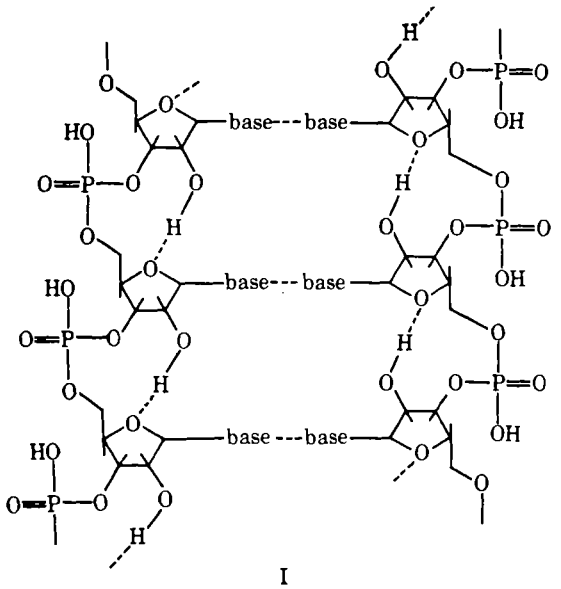
Sir:

In addition to the four main nucleoside constituents (adenosine, cytidine, guanosine, and uridine), *transfer* or *soluble* ribonucleic acids (t-RNA's or s-RNA's) are generally characterized by the presence of a definite amount of methylated nucleosides as minor components (1–10). These methylated nucleosides in t-RNA's are not incorporated as such (11) but are formed at the polynucleotide level by a group of enzymes which catalyze the transfer of methyl groups (methyltransferases, methylases, or transmethylases) from the coenzyme *S*-adenosylmethionine to the t-RNA macromolecules (12–19). The distribution of these methylated units is by no means random and differs in each species (20–22), indicating the existence of certain specificity in the biosynthetic reactions. Viral infection or induction may affect the level of t-RNA methyltransferases (23, 24). It is well known that undermethylated t-RNA's have comparatively inferior aminoacylation activities, codon recognization, and function in protein synthesis (25–29).

It was recently noted that abnormally high levels of methyltransferase enzymes *and* methylase activity, as well as some possible change of specificity of these enzymes, occurred in a variety of neoplastic tissues including virally induced, chemically induced, and spontaneous tumors (30–48). Also reported was the observation that the t-RNA's of many tumors, including both the experimental solid and ascites tumors in animals, as well as human brain tumors, Burkitt lymphoma, glioblastoma, *etc.*, contain highly elevated amounts of methylated—"hypermethylated"—nucleosides (30, 43, 46, 49–52). Since t-RNA's are closely associated with the regulation of protein synthesis at the translation level (26, 53–62) and since alkylating carcinogens were found to alkylate t-RNA to a great extent *in vivo* (43, 63–68) it was postulated that the aberrancy of methyltransferases may be involved in the initiation of tumor induction and neoplasia (32, 66–69). This hypothesis has since received support from other investigators (68–76) and has been considered as one of the most unique and significant findings in cancer research.

Aside from the levels found in neoplastic tissues, larger than normal concentrations of t-RNA methylases were noted in embryonic liver (77) and in chick oviduct (78). Higher t-RNA methylase activity was also observed in fetal brain tissue (79–83). These tissues are characterized by rapid cell division. The activity decreases rapidly in newborn animals after birth (83). It was suggested that the decrease in methyltransferase activity is due to the presence of a methyltransferase inhibitor(s) in adult tissue that is absent in fetal tissue (82, 84). By analogy, it can be postulated that the formation of hypermethylated nucleosides in t-RNA is a result of a lack of methyltransferse inhibitor(s) (85) in the tumor cells. In fact, t-RNA methyltransferase inhibitors, which are found in normal adult rat liver, are virtually absent from the cortex of the highly malignant Walker-256 carcinoma in rats (86). In addition, it was found that an inhibitor prepared from normal adult rat liver had the capacity to inhibit the t-RNA methyltransferase of the Novikoff tumor (82). A search for inhibitors of methyltransferases, therefore, should be of value in cancer chemotherapy, since methylation of t-RNA was shown to be regulated at the enzyme level (87). This is especially true when one considers the possibility that the oncogenic virus might exert its carcinogenicity by introducing a capacity for the synthesis of methyltransferases foreign to the host (15).

Although little is known about actual action of the t-RNA methyltransferase enzymes, information relative to methyltransferase inhibitors may be deduced through an examination of the nature of hypermethylated nucleosides isolated from t-RNA of neoplastic tissues. These nucleosides are composed of the *N*-methylated (*e.g.*, N^6-methyladenosine, N^6-dimethyladenosine, and 1-methylguanosine), the *C*-methylated (*e.g.*, 5-methylcytidine and ribothymine), and the *O*-methylated (*e.g.*, 2′-*O*-methyladenosine, 2′-*O*-methylcytidine, 2′-*O*-methylguanosine, and 2′-*O*-methyluridine) de-



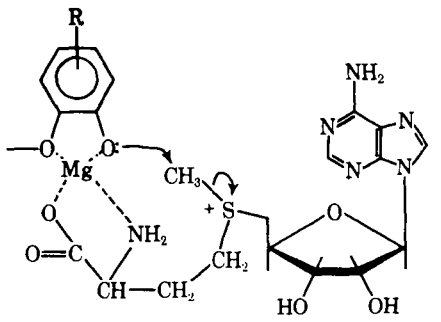
I

rivatives. Among these, studies of the *N*- and the *C*-methylated derivatives have been deservingly conducted, but the significance of the *O*-methylation has received relatively little attention.

Unlike the general structure of DNA, which contains a rather uniform double helical framework, the structure of t-RNA possesses only certain partial double helical regions. The stability of such a helix and base stacking form can be readily achieved by hydrogen bonding of the 2′-hydroxyl group of one ribose with the ring oxygen of a neighboring ribose (88–90), as shown in I.

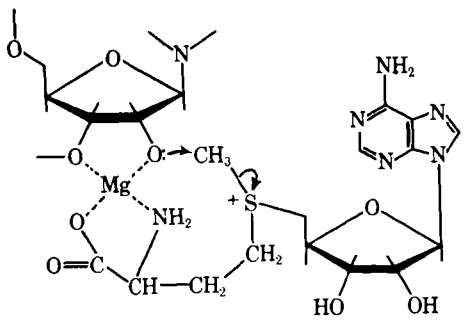
The 2′-hydroxyl group (absent in DNA) in t-RNA is biologically significant in that the aforementioned *endo*-hydrogen bonding modulates the conformation of the secondary and tertiary structures of this macromolecule (75, 90, 91). It is, therefore, logical to assume that aberrant 2′-*O*-methylation in t-RNA drastically alters the *endo*-hydrogen-bonding capabilities of the component nucleosides[1], which may modify the macromolecular structure of t-RNA. This, in turn, would vary the t-RNA binding and recognization specificity during its aminoacylation process and eventually would interfere with normal protein synthesis due to incorrect translation (28, 62, 92), ultimately resulting in abnormal growth. Consequently, agents that inhibit excessive 2′-*O*-methylation (96) or, more specifically, inhibit the action of t-RNA 2′-*O*-methyltransferase should be of value in oncological studies.

Methylation of the 2′-hydroxyl group of ribose requires the enzyme action of certain *aliphatic* *O*-methyltransferase(s). A search of the literature failed to reveal much information on any aliphatic *O*-methyltransferases[2]; on the other hand, *aromatic* *O*-methyltransferases (in the EC 2.1.1 series), such as acetylserotonin-*O*-methyltransferase, catechol-*O*-methyltransferase, hydroxyindole-*O*-methyltransferase, and iodophenyl-*O*-methyltransferase, have been rather extensively studied. Among the latter group, the enzyme catechol-*O*-methyltransferase possesses a unique characteristic in that it requires Mg^{+2} for enzyme action (97). Catechol-*O*-methyltransferase is confined mainly to the *soluble* supernatant fraction of the cell and is found in the liver, kidney, skin, blood cells, granular tissues, and nerve fibers (98). Stoichiometric quantities of catechols, *S*-adenosylmethionine, and Mg^{+2} are required for carrying out enzymic methylation reactions catalyzed by liver catechol-*O*-methyltransferase. Since the activity of t-RNA methyltransferase is enhanced, among others (99), by the presence of small concentrations of univalent and bivalent ions including Mg^{+2} (100), since Mg^{+2} ions were reported to induce secondary structural change of t-RNA (67, 101), and since the distances between the oxygen atoms of the 2,3-dihydroxyl groups of ribosides (about 3.02 Å) and those of the *o*-dihydroxy groups of catechol derivatives (about 3.15 Å) are approximately the same, a complex similar to that proposed for catechol-*O*-methyltransferase (102, 103) (Structure II) can also be proposed for t-RNA 2′-*O*-methyltransferase, as shown in Structure III. Structure II satisfies best the spatial, electronic, and stability requirements for nucleophilic displacement within the complex.



(catalyzed by liver catechol-*O*-methyltransferase)

II



(catalyzed by t-RNA 2′-*O*-methyltransferase)

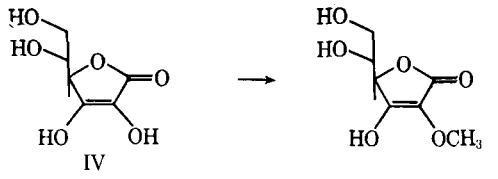
III

A recent report of enzymic methylation of L-ascorbic acid (IV) by rat liver catechol-*O*-methyltransferase (104) substantiates the fact that methylation reactions of this type are by no means limited to six-membered aromatic diols (Scheme I).

The fact that t-RNA's are preferentially methylated may well be due to their solubility and smaller size when compared with other RNA's, thus permitting more

[1] In this regard, the 2′-*O*-methylated riboses somewhat resemble 2′-deoxyriboses of DNA in association characteristics.

[2] A methanol-forming enzyme isolated from the pituitary gland was reported. This enzyme converts water into methanol. *Cf.*, J. Axelrod, in "Transmethylation and Methionine Biosynthesis," S. K. Shapiro and F. Schlenk, Eds., University of Chicago Press, Chicago, Ill., 1965, pp. 71–84.



IV

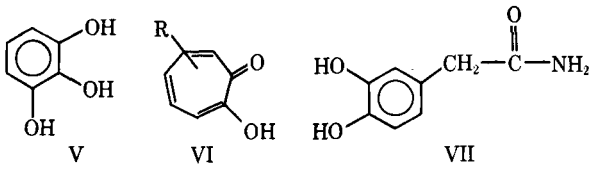
Scheme I

ready access to enzyme sites such as 2′-*O*-methylase. It may be visualized that some inhibitors of catechol-*O*-methyltransferase could also interfere with the 2′-*O*-methylation process of t-RNA. In this regard, it should be pointed out that the reverse inhibition relationship—that certain inhibitors of 2′-*O*-methyltransferase may also interfere with catechol-*O*-methyltransferase—is not necessarily workable. This is due to the fact that the aromatic ring and the dioxy functions in II are coplanar, whereas the corresponding aliphatic hydroxy functions in III are noncoplanar with the tetrahydrofuran ring. Hence, it is relatively easy for the aromatic diols to gain access to the site of 2′-*O*-methyltransferase and difficult for the noncoplanar aliphatic diols to fit in the site of catechol-*O*-methyltransferase.

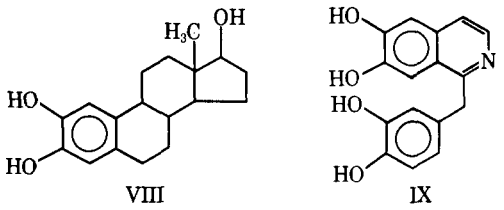
Inhibitors of t-RNA methyltransferases were reported (82, 86, 105, 106) but not characterized. Inhibitors of catechol-*O*-methyltransferase, on the other hand, are widely known. These include pyrogallol (107) (V), tropolones (108) (VI), dopacetamide (VII), 2-hydroxyestradiol (109) (VIII), and papaveroline (IX) (110). These compounds contain at least two *ortho*-substituted oxygenated functions.

The enzyme catechol-*O*-methyltransferase plays an important role in the regulation of phenolic compounds in the metabolism of plants and animals (111). The enzyme catechol-*O*-methyltransferase *per se* has, to our knowledge, not been linked with oncology. Nevertheless, many compounds whose structures are closely related to inhibitors of catechol-*O*-methyltransferase were reported to possess antineoplastic activity. For example, catechol (X*a*), guaiacol (X*b*), protocatechuic acid (X*c*), and certain related compounds have inhibitory action against HF-sarcoma and sarcoma (112); demecolcine[3] (113,114) (XI), a tropolone derivative, and emetine (115) (XII), a reduced isoquinoline derivative, exhibit antileukemic activity.

It, therefore, appears that certain naturally occurring materials or appropriately designed aliphatic or aromatic synthetic compounds having adjacent oxygen functions, or compounds having similar interatomic distances between two oxygen atoms, may interfere with the undesired activity of *O*-methyltransferase in t-RNA and, thereby, inhibit the process of cell proliferation. In the case of aromatic *o*-dihydroxy compounds, the alkoxy (*e.g.*, dimethoxy or methylenedioxy) derivatives may be more suitable than the corresponding hydroxy compounds, since the former are less polar



V VI VII



VIII IX

than the latter and, thus, are more readily transported *in vivo* and absorbed (116). Subsequent *in vivo* cleavage of alkoxy linkages to yield the corresponding hydroxy derivatives should readily provide the desired compounds. Antitumor activity found in certain polysaccharides (117–129), 1,2-dioxo compounds (130–139), and certain aromatic *o*-dialkoxy derivatives (112–115, 140–150) might be related to this type of inhibition (129). The present postulation is compatible with, and complementary to, an earlier antileukemic triangulation pharmacophore hypothesis developed in this laboratory (151).

Experiments pertaining to the aforementioned postulation are being conducted in our laboratory. Results will be reported in the future.

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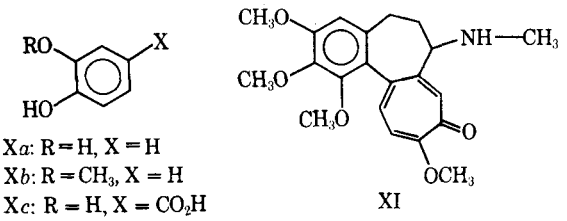
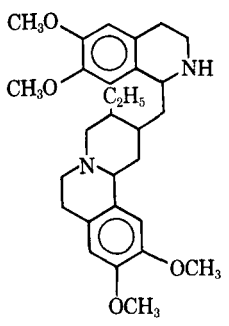


X*a*: R = H, X = H
X*b*: R = CH_3, X = H
X*c*: R = H, X = CO_2H

XI



XII

[3] Demecolcine and related compounds are known to arrest the metaphase of cell division. For the relationship of the metaphase chromosomes and RNA, see, for example: J. Brachet, "Biochemical Cytology," Academic, New York, N. Y., 1957, pp. 149–154; S. Matsui, H. Weinfeld, and A. A. Sandberg, *J. Nat. Cancer Inst.*, **47,** 401(1971); and T. Aya and A. A. Sandberg, *ibid.*, **47,** 961(1971), and references cited therein.

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C. C. CHENG
Midwest Research Institute
Kansas City, MO 64110

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Effect of Complex Formation on Drug Absorption XV: Structural Requirements for Enhancement of Intestinal Absorption of Steroids by *N,N*-Di-*n*-propylpropionamide

Keyphrases ☐ Drug absorption, prednisone, prednisolone—structural requirements for enhancement by *N,N*-di-*n*-propylpropionamide complex formation ☐ Steroid–dialkylpropionamide complexes—structural requirements for formation ☐ Complex formation, prednisone/prednisolone–*N,N*-di-*n*-propylpropionamide—structural requirements ☐ Intestinal absorption, prednisone, prednisolone—structural requirements for enhancement by dialkyl propionamide complex formation

Sir:

N,N-Di-*n*-propylpropionamide (propyl-amide) and certain other substituted propionamides form complexes with prednisone and prednisolone in a lipoid solvent and enhance the transfer of these steroids across intestinal and synthetic lipoid barriers (1–3). The absorption-enhancing effect of propyl-amide appears to involve the formation of a steroid–propyl-amide complex in the barriers. The absorption-enhancing effect is relatively specific, since propyl-amide does not affect the intestinal absorption of several nonsteroid drugs with which it interacts in an organic solvent (4). To explore further the specificity of this effect, the influence of propyl-amide on the absorption of several struc-