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CHEMICAL STUDIES ON THE ORIENTAL PLANT DRUGS—XXIV[1]

STRUCTURE OF GINSENOSIDE-Rg_1, A NEUTRAL SAPONIN OF GINSENG ROOT

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Abstract—Ginsenoside-Rg_1, one of the neutral saponins of Ginseng root, has been purified via its crystalline deca-acetate (IV). The structure of this saponin has been established as 6,20-di-O-β-glucosyl-20*S*-protopanaxatriol (1). Panaxoside A, isolated by Elyakov *et al.* from the same plant is identical with ginsenoside-Rg_1 and the structure of panaxoside A proposed by Elyakov *et al.* is discussed.

IN A PRELIMINARY communication,[2] the structure of ginsenoside-Rg_1, a neutral saponin from Ginseng root was proved to be 6,20-di-O-β-glucosyl-20*S*-protopanaxatriol (1). In the present paper the experimental details which led to the above structure are given and we comment on the formulation of panaxoside A (=ginsenoside-Rg_1) proposed by Elyakov *et al.*[3]

TLC of the crude saponin fraction of Ginseng root revealed the presence of ginsenosides-Ro, a, b_1, b_2, c, d, e, f, g_1, g_2, g_3, and h[4]. Of these, ginsenosides-Rb_1, b_2, and c have been represented by the structure II (the genuine sapogenin: 20*S*-protopanaxadiol (III)).[5a,b]

A mixture of ginsenosides-Rg_1, g_2, and g_3 (tentatively named Rg mixture) was isolated from the methanolic extracts. Ginsenoside Rg_1, colourless semicrystals, m.p. 194–196·5°, was obtained in a pure state via the crystalline deca-O-acetylginsenoside-Rg_1 (IV).

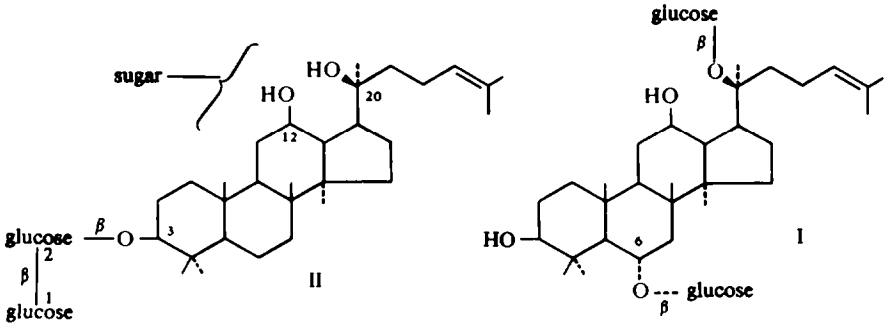


Chart I

On hydrolysis with dilute mineral acid, both the Rg mixture and Rg_1 yielded panaxatriol (V), whose structure has been established to be 6α-hydroxy-20R-panaxadiol.[6] On the other hand, the Rg mixture was hydrolyzed with conc HCl at room temperature, and the sapogenin fraction, which should consist of the chlorides A and B,

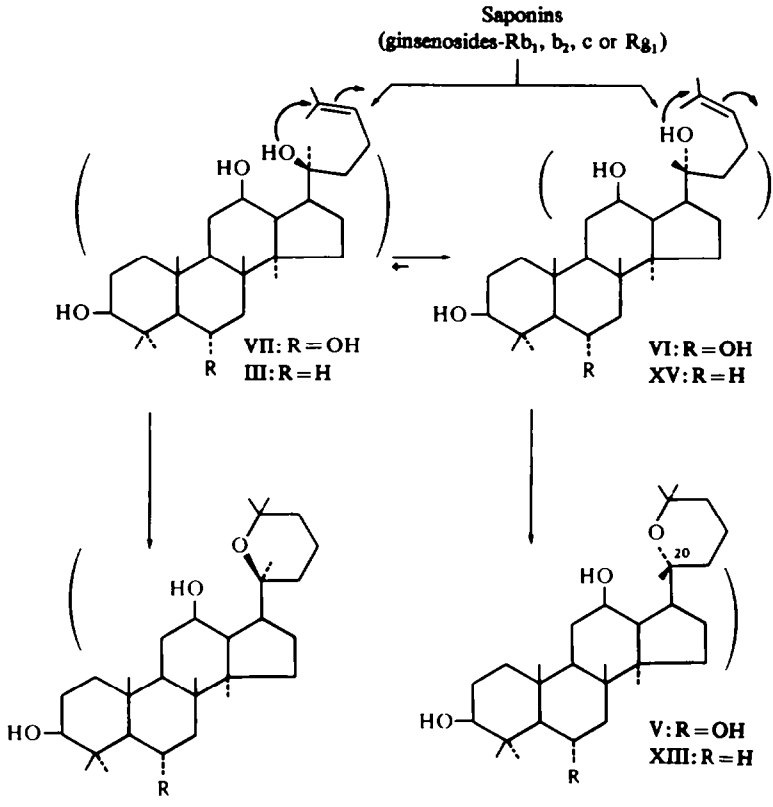


Chart 2

was dehydrochlorinated with tBuOK in DMSO under the conditions used for the preparation of 20*R*-and *S*-protopanaxadiols from ginsenoside-Rb_1, b_2, and c,[5a-c] to give a compound (VI), m.p. 233–235°, together with the compound VII mentioned below. It has been demonstrated that pure Rg_1 gives the same result by the same treatment.

On mild hydrolysis *i.e.*, Smith's degradation[7] or oxidation with periodate followed by alkaline treatment,[8] Rg_1 gave compound VII, as an amorphous powder. In this case, TLC of the reaction mixture revealed no formation of VI.

The compound VI was acetylated to a triacetate (VIII) which still showed an IR absorption of intramolecularly hydrogen bonded OH. On catalytic hydrogenation, VI gave a dihydro-derivative (IX), whose NMR spectrum showed no signals characteristic of a double bond methyls or of an olefinic proton. Treatment of VI with dilute mineral acid under conditions similar to those for the hydrolysis of the saponin afforded panaxatriol (V).[6]

The compound VII, whose NMR spectrum exhibited signals attributable to six quarternary and two allylic methyls in addition to signals due to (—C<u>H</u>—OH eq)$_3$ and an olefinic proton, was subjected to catalytic hydrogenation. The resultant dihydro-compound (X) was oxidized to a triketone (XI), whose ORD curve was almost superimposable on that of the triketone (XII), derived from IX (Fig 1).

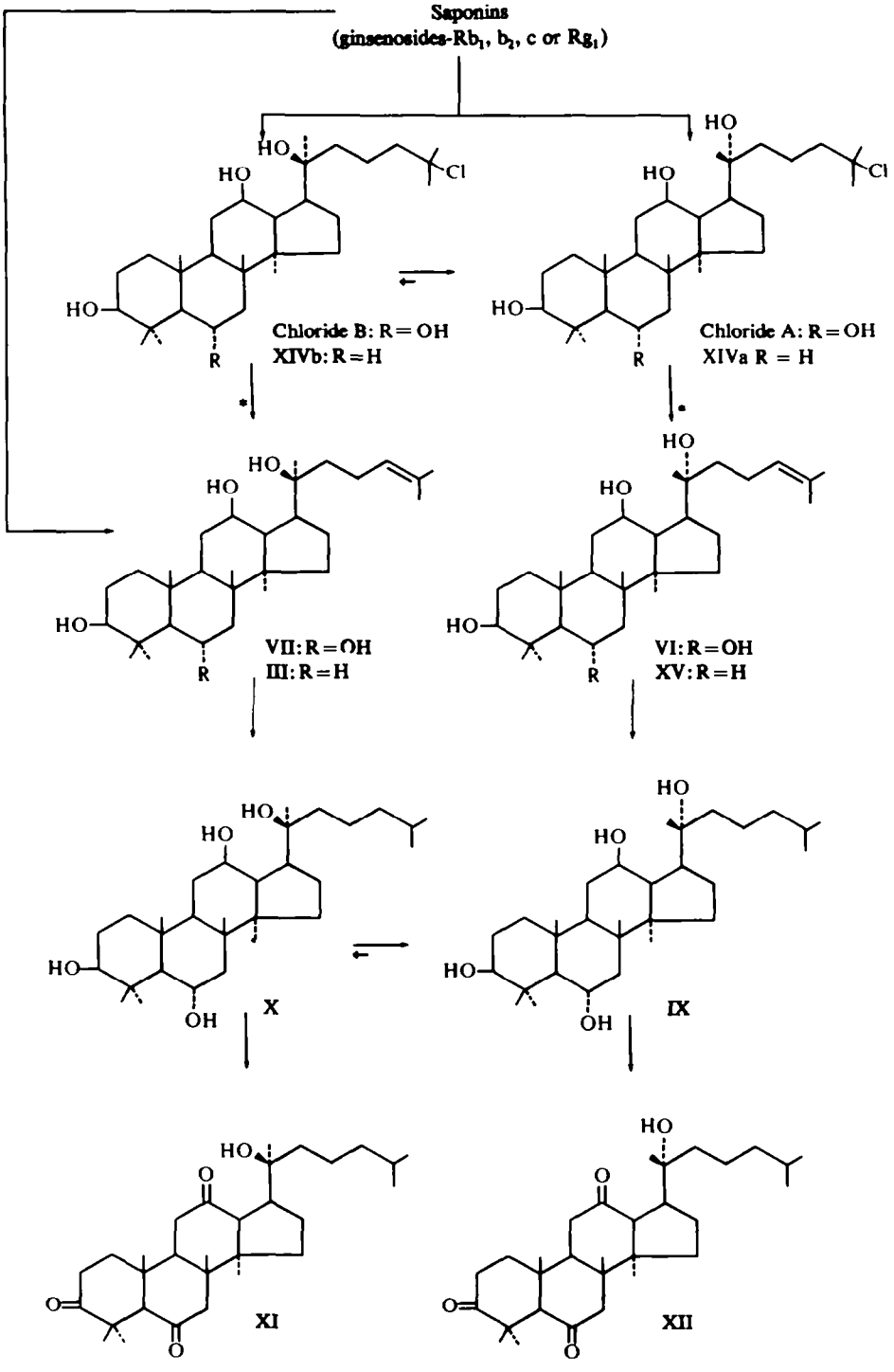

Saponins
(ginsenosides-Rb1, b2, c or Rg1)
Chloride B: R = OH
XIVb: R = H
Chloride A: R = OH
XIVa R = H
VII: R = OH
III: R = H
VI: R = OH
XV: R = H
X
IX
XI
XII

Chart 3

It has already been reported that the hydrolysis of ginsenosides-Rb_1, b_2, and c with dilute mineral acid affords panaxadiol (XIII), whereas their hydrolysis with conc HCl at room temperature gives the chlorides XIVa and XIVb which on dehydrochlorination yield 20*R*- and 20*S*-protopanaxadiols (XV and III), respectively. On the other hand, Smith's degradation of these saponins affords only III, the genuine sapogenin[5a] (Chart 3). The analogy with this as well as the similarity of the properties of VI and VII to those of XV and III led to the conclusion that VI and VII should be represented by a pair of C-20 epimers of 6α-hydroxyprotopanaxadiol, designated now protopanaxatriol, and that the latter compound (VII) should be the genuine sapogenin of Rg_1.

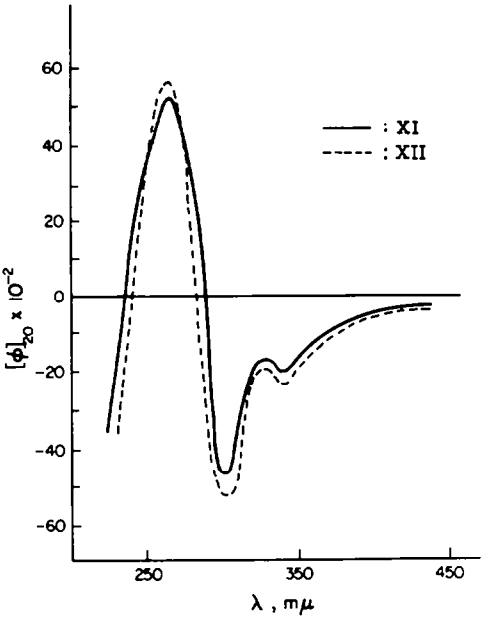


FIG 1

The chirality of C-20 in both VI and VII was established as follows. We have found that the hydroxyl group at C-20 of dammarane-type triterpenes, having a β-hydroxyl group at C-12, easily epimerizes on acid treatment to yield an equilibrated mixture of 20*R*-and 20*S*-epimers with an excess of the former.[5c] Treatment with *p*-toluenesulfonic acid in chloroform or with boiling dilute H_2SO_4 in aqueous ethanol of the dihydro derivative (IX) afforded an equilibrated mixture of IX and X with an excess of IX. This suggests that the chirality of the C-20 of VI and VII is *R* and *S*, respectively. Acid hydrolysis of dihydroginsenoside-Rg_1 (*vide infra*) also gave the same mixture of IX and X. The IR spectra (in CCl_4) of the 12-keto derivatives of dammarane-type triterpenes such as XVIa and b (20*S*) and their C-20 epimers (XVIIa and b, 20*R*) show that the 20*R*-hydroxyl group is mostly intramolecularly H-bonded with the 12-ketone (OH band near 3460 cm^{-1}, and C=O band at 1696 cm^{-1}), while the 20*S*-hydroxyl group is partially H-bonded and exhibits both the free OH band at 3620 and the bonded OH band near 3460 cm^{-1} (C=O bands 1707 and 1697 cm^{-1}). This can be explained in terms of the difference between the conformational stabilities of the H-bonded forms.[9] Fig 2 shows

the OH bands of the triketones XII and XI, indicating that the chirality of C-20 of the former compound (XII) should be assigned as *R* and hence that of (XI) as *S*, though the H-bonded carbonyl band could not be observed in either case owing to the overlapping with the absorption due to the other free carbonyl groups. The names, 20*S*- and 20*R*-protopanaxatriols are now proposed for VII and VI, respectively.

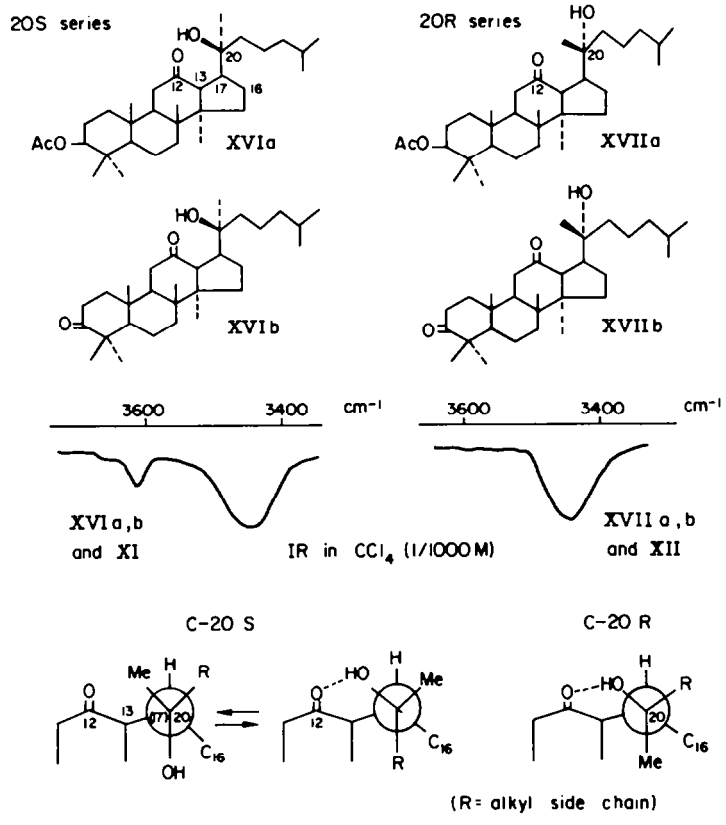
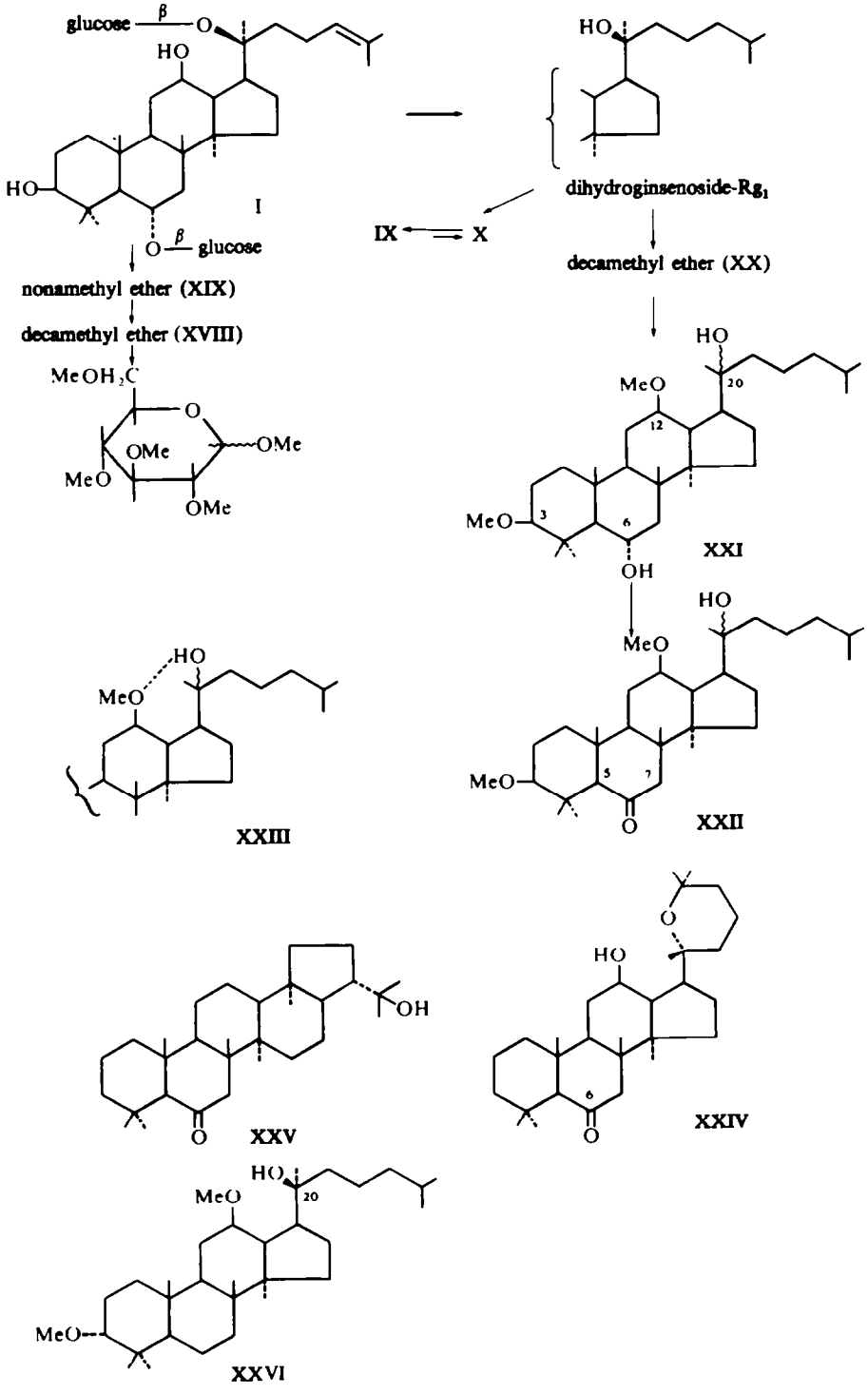


FIG 2

On refluxing with H_2SO_4 in aqueous dioxan, Rg_1 yielded glucose as the sole monosaccharide product, identified by PPC, TLC, and GLC. This observation, coupled with the results of elemental analysis and molecular weight determination of the deca-acetate (IV), indicated that Rg_1 is a diglucoside of 20*S*-protopanaxatriol (VII).

Repeated methylation of Rg_1 with CH_3I and DMSO-NaH reagent[10, 5b] gave an amorphous decamethyl ether (XVIII) via an amorphous nonamethyl ether (XIX). XVIII showed no OH band in its IR spectrum and exhibited NMR signals due to $(—OCH_3)_{10}$ (singlets); XIX showed an OH band at 3463 cm^{-1} in CCl_4 and exhibited NMR signals due to $(—OCH_3)_9$ (singlets). XVIII was subjected to methanolysis with HCl in methanol. GLC of the sugar moiety of the products indicated the presence of methyl 2,3,4,6-tetra-O-methylglucopyranoside, excluding the formation of methyl tri- or di-O-methylglucopyranoside. The β-linkage of both the glucosyl moieties of Rg_1 was revealed by the coupling constants of the anomeric proton signals (doublets, IH each) at

CHART 4


glucose β O
HO
HO
I
O β glucose
nonamethyl ether (XIX)
decamethyl ether (XVIII)
MeOH2C
OMe
OMe
MeO
OMe
HO
dihydroginsenoside-Rg1
IX ⇌ X
decamethyl ether (XX)
HO
MeO
20
12
MeO
3
6
OH
XXI
HO
MeO
MeO
5
7
O
XXII
HO
MeO
XXIII
OH
O
XXV
O
HO
6
O
XXIV
HO
MeO
20
MeO
XXVI

δ 4·24 (J = 7 Hz) and 4·39 (J = 7 Hz) in the NMR spectrum of XVIII and at δ 4·27 (J = 7·5 Hz) and 4·46 ppm (J = 7·5 Hz) in that of XIX. Consequently, it follows that each of the β-glucosyl moieties of Rg_1 is combined separately with two of the four hydroxyl groups of the sapogenin, 20*S*-protopanaxatriol (VII).

The dihydroginsenoside-Rg_1 was methylated to a decamethyl ether (XX); IR has no OH band. Hydrolysis of XX with conc HCl at room temperature and subsequent chromatography of the resulted genin fraction gave 3,12-di-O-methyl-20ξ-protopanaxatriol (XXI). The location of two of the methoxyl groups of XXI was established by the following evidences. Oxidation of XXI afforded a ketone (XXII), whose IR absorption bands (in CCl_4) at 3340 (strong, concentration-independent, intramolecularly H-bonded OH) and 1713 cm^{-1} (C=O) were consistent with the partial structure XXIII, eliminating the possibility of a 12-ketone, which would be expected to exhibit H-bonded OH and C=O bands near 3460 and 1695 cm^{-1}, respectively (*vide supra*). It has already been found that the IR spectra of the 20-hydroxy-12β-methoxy derivative in CCl_4 shows a strong OH band due to intramolecular H-bonding. The ORD curve of XXII showed a negative Cotton effect which was quite similar to that of the 6-keto derivative (XXIV) prepared from panaxatriol (V) and was clearly different from those of the 12-keto derivative (XVIIa) and the 3-keto derivatives of dammarane-type triterpenes, of which the latter have been reported to show positive Cotton effects[11] (Fig 3). The negative Zimmerman test from XXII also excluded the presence of the 3-ketone.[12] The assignment of the structure of XXII was further secured by its NMR spectrum. The signals due to the active methylene (C-7) and the methine (C-5) protons of the 6-keto derivative (XXIV) have been defined by the deuterification procedure and the comparison with those of zeorinone (XXV).[6] In the NMR spectrum of XXII, the active proton signals appeared at similar positions with similar coupling patterns (a pair of doublets at δ 1·87 and 2·60 ppm (1H each, J = 11 Hz) and a singlet at δ 2·11 ppm (1H)) to those of XXIV

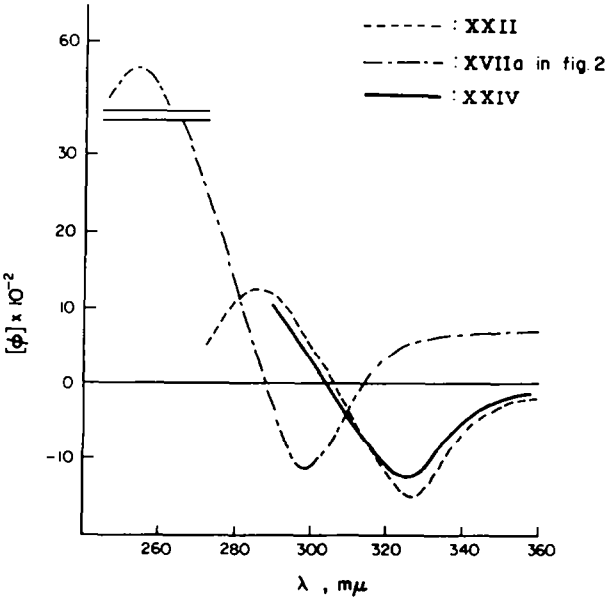


Fig 3

and XXV. Since the equilibrated epimerization of the C-20 hydroxyl group[5c] has also been observed in the acid treatment of 3,12-di-O-methylbetulafolianetriol (XXVI),[5d] the absolute configuration of the C-20 hydroxyl group, of the dimethyl ether (XXI) isolated in the present study cannot at present be established.

On the basis of all of the above evidence, it can be concluded that the hydroxyl groups at C-3 and -12 of the sapogenin moiety of Rg_1 should be free and consequently that two β glucosyl moieties must be combined with the hydroxyl groups at C-6 and -20. Rg_1 should be thus represented as 6,12-di-O-β-glucosyl-20*S*-protopanaxatriol (1). The absence of an OH band in the IR spectra of the deca-acetate (IV) and decamethyl ethers (XVIII and XX) also supports the presence of an O-glucosyl linkage at C-20, since a C-20 hydroxyl group of this type has been found to resist acetylation and methylation and hence an OH band should be observed in Rg_1 even after acetylation or methylation, if the free hydroxyl group is present at C-20.

Ginsenoside-Rg_1 is the first example of a saponin of the dammarane-type whose structure has fully been established. Elyakov *et al.* have also reported the isolation of saponins from Ginseng root cultivated in the Far Eastern region of the USSR and designated them panaxosides A-F.[13] Of these saponins, panaxoside A yielded glucose and panaxatriol (V)[6] on acid hydrolysis. Comparisons of the physical and chemical properties and the TLC of Rg_1 and its crystalline deca-acetate (IV) with those of panaxoside A and its acetate as well as the comparison of the X-ray powder pattern of IV with that of the panaxoside A acetate proved the identity of these saponins.

Elyakov *et al.*[3] have proposed the formula XXVII for the genuine sapogenin of panaxoside A without referring to our report[5c] concerned with the acid catalyzed epimerization of the C-20 hydroxyl group. However, all of the chemical reactions and spectral data described in Elyakov's report can be more reasonably explained by our present formulation of Rg_1 when the acid catalyzed epimerization of the C-20 hydroxyl group is considered. The NMR signals characteristic of the C-methyl groups of the deca-acetate (IV) measured at both 100 Mc and 60 Mc in pyridine-d_5 excluded conclusively the presence of a secondary methyl group ($—C_{(20)}H—C\underline{H}_3$). This cannot be in agreement with Elyakov's formula (XXVII).

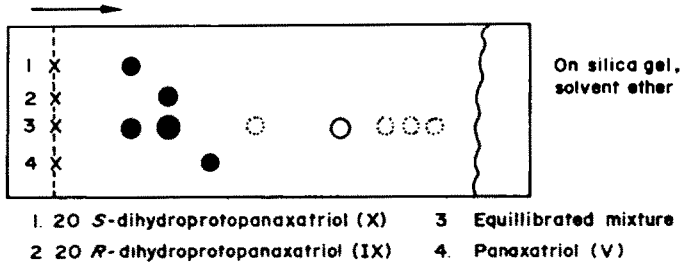
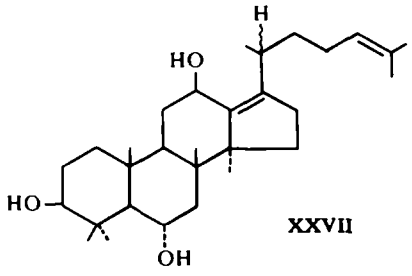


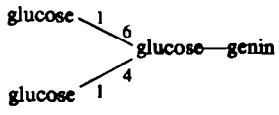
FIG. 4.

Elyakov *et al.* also reported that panaxoside A is a triglucoside and that the sugar moieties are linked with the sapogenin as shown in XXVIII.[3b] However, careful examination by GLC using an authentic sample of methyl 2,3-di-O-methylglucoside (XXIX) as a reference revealed the absence of XXIX from the products of the methanolysis of the decamethyl ether (XVIII). This is inconsistent with their formula-

tion. The molecular weight determination and mass spectrum of the deca-acetate (IV)[14] as well as the NMR signals of the anomeric protons of XVIII and XIX are also incompatible with their structure.



XXVII



XXVIII

EXPERIMENTAL*

Extraction and separation of ginsenosides-Rg (I). The powdered Ginseng (Japanese name Hakusan, 2·0 kg) was repeatedly extracted with boiling MeOH and the combined extract was concentrated to dryness *in vacuo* to give a syrupy residue, which was treated with cold MeOH (3l) to remove less soluble substances. The MeOH soln was then concentrated to 800 ml *in vacuo* and poured into acetone (3l) to deposit the more polar saponins, ginsenosides-Ra, b, c, d. The supernatant was evaporated to dryness and the residue dissolved in H_2O (1l). The aq soln was washed with $CHCl_3$ and then extracted several times with a $CHCl_3$-MeOH mixture (2 : 1). The combined organic layers afforded a brownish powder (8·0 g) after evaporation, which was chromatographed on silica gel (graduent elution with $CHCl_3$, $CHCl_3$: MeOH—95 : 5 then 90 : 10) to separate a mixture of ginsenosides-Rg (Rg mixture) (3·4 g) from other substances. The separation was followed by TLC on silica gel using $CHCl_3$: MeOH : H_2O (65 : 35 : 10).

Deca-O-acetylginsenoside-Rg$_1$ (IV). Rg mixture (1·03 g) was acetylated with Ac_2O (20 ml) and pyridine (40 ml) at room temp overnight. The reaction mixture was poured into ice water and the ppt was taken up in ether. The ethereal soln was washed with water repeatedly and concentrated to dryness. The residue was recrystallized from a $CHCl_3$–EtOH mixture giving deca-O-acetylginsenoside-Rg_1 (IV) (620 mg) as colourless needles, m.p. 242–243°, $[\alpha]_D^{25}$ + 7·0 ($CHCl_3$). (Calc. for $C_{62}H_{92}O_{24}$ C, 60·97. H, 7·59. Found C, 60·93; H, 7·39%. Molecular weight: Calc. 1221·4. Found 1199·1 (osmometric method, in $CHCl_3$ at 37°). IR: (in CCl_4) strong OAc absorption and no OH band. NMR: (in $CDCl_3$)— $\overset{|}{\underset{|}{C}}$—$CH_3$ δ 0·96(9H), 1·03(3H), 1·05(3H), 1·18(3H) (all singlets), —$\overset{|}{C}$=$\overset{|}{C}$—CH_3 1·59, 1·65 (singlets, 3H each), —$OCOCH_3$ 1·96(3H), 1·98(9H), 2·01(9H), 2·03(6H), and 2·09 ppm (3H) (all singlets): (in pyridine-d_5)—$\overset{|}{\underset{|}{C}}$—$CH_3$ δ 0·97(3H), 1·02(3H), 1·14(3H), 1·25(3H), 1·29(3H), 1·42(3H), —C=C—CH_3 1·68(6H) (all singlets)— at 100 mc as well as 60 mc.

Ginsenoside-Rg$_1$. A soln of IV in 5% MeOH/KOH was refluxed for 2 h. After concentration and addition of water, the reaction mixture was extracted with nBuOH. The BuOH layer was washed with water and evaporated *in vacuo* to dryness affording Rg_1 as a colourless powder (330 mg) which was soluble in MeOH, pyridine and hot Me_2CO and less soluble in EtOAc and $CHCl_3$. Recrystallization from nBuOH-methyl ethyl ketone gave colourless semi-crystals, m.p. 194–196·5°, $[\alpha]_D^{19}$ + 32° (pyridine). (Calc for $C_{42}H_{72}O_{14}.2H_2O$: C, 60·26, H, 9·15. Found C, 60·11, H, 9·42%).

Hydrolysis of Rg$_1$. (a) With dil H_2SO_4 in aqueous dioxan: A soln of Rg_1 (10 mg) in N—H_2SO_4 (dioxan : H_2O 3 : 1) (2ml) was refluxed for 2·5 h. The reaction mixture was diluted with water and washed with $CHCl_3$ and nBuOH. The aqueous layer was deionized by passing through a column of ion exchange resin (IRA-4B) and concentrated. Glucose was detected by TLC, PPC, and GLC.

* All m.p.s. were determined on a Kofler block and remain uncorrected. NMR spectra were taken at 100 Mc in $CDCl_3$ soln with TMS as internal standard, unless otherwise stated.

(b) With dil H_2SO_4 in aqueous EtOH: Rg_1 was hydrolyzed by refluxing with 5% H_2SO_4 in 50% aq EtOH for 6 h. The soln was diluted with water and the resulted ppts were taken up in ether. The hydrolysate was chromatographed on silica gel to give panaxatriol (V) (=20*R*-panaxatriol),[6] as colourless needles from benzene, m.p. 238–238·5°.

(c) Smith's degradation (formation of 20*S*-protopanaxatriol (VII):[7] To a cooled soln of Rg_1 (2 g) in water (400 ml) containing a little MeOH, $NaIO_4$ (3 g) was gradually added with stirring. The reaction mixture was kept at 4° with stirring for 4 days. The resulting ppt was collected, washed with water and dissolved in warm EtOH–H_2O (2:1, 240 ml). After cooling, $NaBH_4$ (2 g) was added to the soln, which was then kept at room temp overnight with stirring. The reaction mixture was acidified to pH 2 by careful addition of dil H_2SO_4 and allowed to stand at room temp for 2 days with stirring. The EtOH was removed *in vacuo* at low temp and the aqueous mixture was extracted with ether. The ethereal layer was washed with water and concentrated to dryness. The residue was chromatographed on silica gel (graduent elution with $CHCl_3$, $CHCl_3$:Et_2O = 9:1 then 8:2) to give 20*S*-protopanaxatriol (VII), an amorphous powder from benzene. This has not yet been obtained as crystals but is shown as homogeneous by TLC and NMR spectrum. (VII): $[\alpha]_D^{21}$ + 42·9 ($CHCl_3$); ν_{max} 3620 (free OH) and 3414 cm^{-1} (concentration independent, intramolecularly H-bonded OH); NMR δ 0·93(6H), 0·98(3H), 1·06(3H), 1·13(3H), 1·18(3H), 1·62(3H), 1·68(3H) (all singlets), 3·18 (1H broad), 3·58 (1H broad), 4·13 (1H broad) and 5·15 ppm (1H broad).

(d) Oxidation with periodate followed by alkaline treatment[8]: Rg_1 (500 mg) was oxidized with $NaIO_4$ (1·5 g) in water under the same condition as Smith's degradation. The reaction mixture was extracted with nBuOH and the BuOH layer was washed with water and then concentrated to dryness below 60° under reduced pressure. The residue was dissolved in 5% aq KOH (50 ml) and the soln was refluxed under N_2 for 2 h. The reaction mixture was cooled and extracted with ether. The TLC of the crude product (on silica gel, solvent: ether) revealed the presence of VII and absence of VI as in the case of Smith's degradation.

The triketone (XI) *from* 20*S-protopanaxatriol* (VII) *through* 20*S-dihydroprotopanaxatriol* (X). VII (65 mg) was hydrogenated in the presence of Adams catalyst in an AcOH–AcOEt mixture to give X, which is amorphous but shown as homogeneous by TLC, and lacks the NMR signals characteristic of the olefinic proton and the allylic methyl groups.

To a soln of X (65 mg) in Me_2CO (8 ml) was added Jones's reagent (0·5 ml) and the soln was allowed to stand at room temp for 6 h. On working up in the usual manner followed by recrystallization from Me_2CO, the product afforded XI as colourless needles, m.p. 160–163°, $[\alpha]_D^{34}$ + 13° (MeOH). (Calc for $C_{30}H_{48}O_4$: C, 76·22, H, 10·24. Found: C, 76·06; H, 10·31%)IR $\nu_{max}^{CCl_4}$ 3616 (free OH), 3461 (concentration independent, intramolecularly H—bonded OH), and 1717 cm^{-1} (C=O) (see Fig 2).

20*R-Protopanaxatriol* (VI). Rg mixture (11g) was dissolved in conc HCl (55 ml) and the soln kept at room temp for 22 h with occasional shaking. The reaction mixture was diluted with water and extracted with ether. The ethereal layer was washed with water, dried, and evaporated to dryness to give a brownish residue (3·8 g), a mixture of the chlorides A and B which was positive to a Beilstein test. The mixture of the chlorides (3 g) was dissolved in DMSO (160 ml) and to this soln was added tBuOK (3·3 g) in DMSO (260 ml) and the mixture heated at 75° for 4 h. After dilution with water and subsequent extraction with ether, the resulted ether layer was washed with water several times to remove DMSO, dried, and evaporated. The TLC (on silica gel, solvent: ether) showed the formation of both 20*S*- and 20*R*- protopanaxatriols (VII and VI) with an excess of the latter compound. The R_f value of the TLC (silica gel, solvent: ether) of VI is higher than that of VII. Column chromatography of the crude products on silica gel (solvent: $CHCl_3$ and then $CHCl_3$:Et_2O 9:1) afforded VI (330 mg) as colourless needles from $CHCl_3$, m.p. 233–235°, $[\alpha]_D^{16}$ – 8·2° (MeOH) (Calc for $C_{30}H_{50}O_4$: C, 75·58; H, 11·00. Found: C, 75·40; H, 10·86.) IR $\nu_{max}^{CCl_4}$ 3470 and 3240 cm^{-1} (OH); NMR (in pyridine-d_5) δ 1·0(6H), 1·17(3H), 1·38(3H), 1·41(3H), 1·64(6H), and 1·90 ppm (3H), all singlets. The anomalous shift of C-4 methyl signals of the triterpenes having α-hydroxyl group at C-6 in pyridine will be reported elsewhere in relation to the structural study of V. Like 20*R*-protopanaxadiol (XV), VI is less soluble in organic solvents and more easily crystallized than its 20*S*-isomer (VII).

It has been found that pure Rg_1 also affords the same mixture of VI and VII by hydrolysis with conc HCl followed by treatment with base under the same conditions as above.

Panaxatriol (V) *from* 20R-*protopanaxatriol* (VI). VI(20 mg) was heated under reflux with 5% H_2SO_4 in 50% aqueous EtOH for 5·5 h. The crystals deposited during the reaction were collected and recrystallized from benzene to give V, which was identified by comparison with an authentic sample.

Acetylation of 20*R-protopanaxatriol* (VI). A solution of VI (100 mg) in Ac_2O (2 ml) and pyridine (4 ml) was allowed to stand at room temp overnight. Working up of the product in the usual way gave 3,6,12-tri-O-acetyl-20*R*-protopanaxatriol (VIII), which was difficult to be crystallized but homogeneous by TLC (silica

gel, solvent: AcOEt: cyclohexane: H_2O 30:70:0·1), $[\alpha]_D^{16}$ + 10·9° ($CHCl_3$); IR $\nu_{max}^{CCl_4}$ 3540 (concentration independent, intramolecularly H-bonded OH) and 1736 cm^{-1} ($OCOCH_3$); NMR δ 0·91 (3H), 1·00 (9H), 1·10 (3H), 1·14 (3H), 1·60 (3H), 1·65 (3H) and 2·04 ppm (9H) all singlets: (in pyridine) δ 0·89 (3H), 0·98 (6H), 1·14 (6H), 1·26 (3H), 1·60 (3H) and 1·68 ppm (3H) all singlets.

20R-Dihydroprotopanaxatriol (IX). Hydrogenation of VI (150 mg) in EtOH (8 ml)-AcOH (1·5 ml) mixture using Adams catalyst gave, colourless needles from $CHCl_3$, m.p. 273–274°, $[\alpha]_D^{16}$ – 14° (MeOH) Calc for $C_{30}H_{54}O_4$; C, 75·26; H, 11·37. Found: C, 75·27; H, 11·31%) negative to nitromethane test; NMR (in pyridine-d_5) no signals characteristic of the olefinic proton and the allylic methyl groups.

The triketone (XII) *from* 20R-dihydroprotopanaxatriol (IX). To a solution of IX (34 mg) in acetone was added Jones' reagent (0·4 ml) and the reaction mixture was kept at room temp overnight. After addition of a small amount of MeOH to decompose the excess reagent, the mixture was filtered. The filtrate was diluted with water and extracted with ether. The ethereal layer was washed with water, 2N Na_2CO_3 and water, successively dried, and evaporated to dryness. The residue was recrystallized from MeOH–H_2O mixture to give XII, colourless needles, m.p. 167–168°, $[\alpha]_D^{32}$ 0° (MeOH). Calc for $C_{30}H_{48}O_4$; C, 76·22; H, 10·24: Found C, 76·10, H, 10·31%) IR $\nu_{max}^{CCl_4}$ 3466 (concentration independent, intramolecularly H-bonded OH), and 1715 cm^{-1} (C=O).

Deca-O-acetyldihydroginsenoside-Rg$_1$. Deca-O-acetylginsenoside-Rg_1 (IV)(120 mg) was hydrogenated with Adams catalyst in dioxan (11 ml)-AcOH (1·2 ml) at room temp to consume 1 mole equiv of H_2. The catalyst was removed by filtration and the filtrate was concentrated at low temp *in vacuo* to dryness. The residue was crystallized from $CHCl_3$-MeOH affording deca-O-acetyldidhydroginsenoside-Rg_1, as colourless needles (80 mg), m.p. 258–260°, $[\alpha]_D^{21}$ + 3·1° ($CHCl_3$) (Calc for $C_{62}H_{94}O_{24}$: C, 60·87; H, 7·75. Found: C, 61·07; H, 7·58%) negative to tetranitromethane test; IR (in CCl_4): No OH band; NMR: No signals due to the olefinic and allylic methyl protons.

Dihydroginsenoside-Rg$_1$. (1) Deca-O-acetyldihydroginsenoside-Rg_1 (60 mg) was saponified by refluxing 5% MeOH/KOH (7 ml) for 2 h. After concentration and subsequent addition of water, the mixture was extracted with nBuOH. The BuOH extract was washed with water and evaporated to dryness giving dihydroginsenoside-Rg_1, colourless powder, $[\alpha]_D^{24}$ + 17·0 (pyridine); NMR: No signals due to the olefinic and allylic methyl protons, which was difficult to crystallized but homogeneous by TLC (silica gel, $CHCl_3$: MeOH: H_2O = 65:35:10). (2) Rg_1 was hydrogenated with Adams catalyst in MeOH–AcOH to give dihydroginsenoside-Rg_1. The identity of this dihydrosaponin with that obtained from the dihydrodecaacetate was confirmed by comparison of TLC, optical rotation, and NMR spectra

Acid catalyzed epimerization of 20R-*dihydroprotopanaxatriol* (IX). (a) With 5% H_2SO_4: IX (5 mg) was refluxed with 5% H_2SO_4 in 50% aq EtOH (3 ml) for 5 h. The soln was diluted with water and extracted with ether. The TLC (silica gel, solvent; ether) of the ethereal extract is illustrated in Fig 4. (b) With *p*-toluenesulfonic acid: IX (1 mg) was treated with TsOH (1 mg) in $CHCl_3$ (0·2 ml) saturated with water at 30–34° and the epimerization process was followed by TLC (silica gel, solvent: ether). After 2 h the formation of X began to be observed and it took approx 2 days to obtain the final equilibration of IX and X.

Acid hydrolysis of dihydroginsenoside-Rg$_1$. Dihydroginsenoside-Rg_1 was subjected to hydrolysis by refluxing with 5% H_2SO_4 in 5% aq EtOH for 5 h. The TLC (Fig 4) of the crude hydrolysate indicated the presence of the equilibrated mixture of IX and X, the former of which was further purified by column chromatography on silica gel being identified as crystalline state. In this case, no formation of panaxatriol (V) was secured.

Methylation of dihydroginsenoside-Rg$_1$. Commercial NaH (50%, 2·1 g) was washed with light petroleum and heated with DMSO (60 ml) at 60–75° under N_2 with stirring for 1 h. To this reagent was gradually added a soln of dihydroginsenoside-Rg_1 (2 g) in DMSO (50 ml) at room temp and the mixture was stirred at room temp for 2½ h. After addition of CH_3I (45 g), the mixture was allowed to stand at room temp for 2 days. The entire reaction was carried out under N_2. The reaction mixture was then diluted with water and extracted with ether. The ethereal layer was washed with water repeatedly and dried. The residue was methylated again under the same conditions as above and the products were chromatographed on silica gel to give the decamethyl ether(XX), which was amorphous but proved to be homogeneous on TLC (silica gel, solvent; $CHCl_3$: ether = 1:1) and exhibited NMR signals characteristic of ten O-methyl groups. IR spectrum of XX showed no OH absorption band.

Hydrolysis of XX. XX (1 g) was dissolved in conc HCl and the soln allowed to stand at room temp for 7 h. The reaction mixture was diluted with water and extracted with ether. The ethereal layer was washed with water, dried, and evaporated to dryness. Column chromatography of the residue on silica gel followed by recrystallization from MeOH gave 3,12-di-O-methyl-20ξ-dihydroprotopanaxatriol (XXI) (150 mg) as

colourless plates, m.p. 180–182°; IR $\nu_{max}^{CCl_4}$ 3620 (free OH) and 3380 cm^{-1} (concentration independent. bonded OH; $[\alpha]_D^{11}$ + 19·3° ($CHCl_3$) (Calc for $C_{32}H_{58}O_4$): C, 75·84; H, 11·54. Found: C, 75·89; H, 11·49%). NMR δ 3·36 ppm (6H, singlet, (—O—CH_3)$_2$).

Oxidation of 3,12-*di*-O-*methyl*-20ξ-*dihydroprotopanaxatriol* (XXI). To a soln of XXI (120 mg) in acetone (20 ml) was added Jones' reagent (0·5 ml) and the soln kept at room temp for 3 h. After being worked up in the usual way, the product was recrystallized from aq MeOH, giving the ketone (XXII), colourless needles, m.p. 155–156°, $[\alpha]_D^{14}$ − 6·2° ($CHCl_3$) (Calc for $C_{32}H_{56}O_4$: C, 76·14; H, 11·18. Found: C, 76·30; H, 10·93%).

Methylation of Rg_1 Rg_1 (0·5g) was methylated with NaH (50%, 0·6 g) in DMSO (10 ml) and CH_3I (4 ml) in DMSO (8 ml) as for the methylation of dihydroginsenoside-Rg_1. The crude reaction products were purified by column chromatography on silica gel to give the amorphous decamethyl ether (XVIII); IR (in CCl_4) no OH band; NMR δ 0·90 (3H), 0·95 (3H), 0·98 (3H), 1·02 (3H), 1·19 (3H), 1·33 (3H), 1·63 (3H), 1·69 (3H), 3·23 (3H), 3·35 (3H), 3·36 (6H), 3·51 (6H), 3·55 (3H), 3·56 (3H), 3·62 (3H), 3·63 (3H) all singlets, and 5·06 ppm (1H broad, olefinic proton) along with the amorphous nonamethyl ether (XIX); IR $\nu_{max}^{CCl_4}$ 3463 cm^{-1}; NMR δ 0·93 (3H), 1·00 (6H), 1·07 (3H), 1·36 (6H), 1·62 (3H), 1·71 (3H), 3·38 (9H), 3·53 (6H), 3·59 (6H), 3·62 (3H), 3·56 (3H) (all singlets), and 5·11 ppm (1H broad).

Methanolysis of the decamethyl ether (XVIII). A suspension of XVIII (30 mg) in 5% MeOH HCl (3 ml) in a sealed tube was heated in a boiling water bath for 6 h. The reaction mixture was deionized by passing through a column of IRA-4B and concentrated. GLC of the product (column 12% DEGS, column temperature 167°) revealed the presence of methyl 2,3,4,6-tetra-O-methylglucoside. In order to re-examine Elyakov's work, GLC of the reaction product using the authentic sample of methyl 2,3-di-O-methylglucoside as a reference was carefully carried out, excluding the presence of even a trace of this compd.

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